PROCEEDINGS.

VOL. 29. OCTOBER, 1931. No. 1.

Pacific Coast Section.

California Institute of Technology, June 16, 1931.

5695

Effect of Adrenalectomy on the Testes of the Rat.

S. C. FREED, B. BROWNFIELD, AND HERBERT M. EVANS.
From the Department of Anatomy, University of California.

In the literature, clinical observations are the most prominent in dealing with the relationship between the adrenals and the sex organs. There are many cases on record of precocious maturity, virilism, hermaphroditism, and other sex disturbances associated with adrenal pathology.[1] Most of the work on laboratory animals demonstrates changes in the adrenals due to castration,[2] pregnancy,[3] and ovulation.[4] The effect of adrenalectomy in suppressing the estrous cycle in rats is of little significance since the altered metabolism easily obscures any specific relationship.[5] Novak[6] found that the testes of adrenalectomized rats contained degenerated tubules, the younger rats being more susceptible to this condition than the older ones. MacMahon and Zwemer[7] noticed only changes in the interstitial cells of the cat testes. Jaffe[8] in working with large numbers of rabbits concluded that tubular degeneration occurs in only a small percentage of cases and that it is due to the general poor health

1 Barker and Hoskins, *Endo. and Metab.*, 1922, **2**, 345.
2 Altenberger, *Pflüg. Arch.*, 1924, **202**.
3 Donaldson, *Am. J. Phys.*, 1924, **68**, 517.
4 Riddle, *Am. J. Phys.*, 1923, **66**, 322.
5 Weyman, *Am. J. Phys.*, 1928, **86**.
6 Novak, *Arch. f. Gynäk.*, 1913, **101**, 36.
7 MacMahon and Zwemer, *Am. J. Path.*, 1929, **5**, 491.
8 Jaffe, *J. Exp. Med.*, 1923, **38**, 107.

of the animals. Feeding adrenal cortex is said to increase the size of rat and chick testes.[9, 10]

One is impressed, from Britton's recent review,[11] by the lack of uniformity in the results of numerous experiments on the effects of adrenalectomy. This is especially noticeable in the reports on the survival times of rats, a point of fundamental importance. Workers of much experience have for years stated that adrenalectomized rats live on for long periods due to the possession of accessory cortical tissue. In the hands of different experimenters, from 20 to 80% of the rats are able to withstand the operation successfully. In our first group of operations we found that about one-quarter of the animals seemed unaffected by having their adrenals removed, an outcome which was expected. Later in excising the gland, we detached with them one-quarter inch of the pedicle, the result being that there were no survivals. The immature males died regularly in 4 to 6 days after extirpation of their adrenals. Mature males in a smaller series lived on the average a day or two longer. Pencharz, Giragossintz and Olmsted[12] have shown convincingly that a minute fragment of cortical tissue is capable of regeneration which permits survival indefinitely. This explains the high percentages of animals which fail to develop adrenal insufficiency. In our experiment there were used only animals which displayed the symptoms of acute insufficiency.

The rats, after adrenalectomy, were observed continually until death was obviously near; they were then killed, the testes were weighed and fixed immediately. On gross examination, the testes were pale, soft, and edematous. In the immature rats, they were considerably lighter than their controls (Table I); the mature rats were unaffected in this respect. Histologically, the spermatic tubules were ragged, fragmented, and disorganized; clumps of cells lay in the lumina and often only the germinative layer remained in position. In the adult testes the spermatocytes were severely affected, staining ghost-like with eosin. The mature testis undergoes as great a degeneration as the immature, contrary to Novak's observations.

We offer here some evidence which indicates that this effect on the testes is not through any action on the hypophysis. Smith has conclusively shown that there is a direct relationship between the adrenals and the anterior lobe.[13] Hypophysectomy causes an atrophy

[9] McKinley and Fisher, *Am. J. Phys.*, 1926, **76**, 268.

[10] Hoskins and Hoskins, *Arch. Int. Med.*, 1916, **17**.

[11] Britton, *Phys. Rev.*, 1930, **10**.

[12] Pencharz, Giragossintz and Olmsted, *Science*, 1930, **72**, 175.

[13] Smith, *J. Am. Med. Assn.*, 1927, **88**, 158.

TABLE I.

Adrenalectomized Males			Controls
Age at Operation in days	Age at Death in days	Weight of Testes in gm.	Weight of Testes in gm.
26	29	.320	.405
26	29	.360	.410
26	29	.430	.440
26	30	.490	
26	30	.305	.530
26	30	.340	.605
26	30	.340	.620
26	30	.400	.560
26	30	.410	.650
26	30	.450	
26	30	.410	.520
26	30	.480	
27	31	.345	
26	32	.690	.320
27	33	.370	.710
27	33	.335	.700
27	33	.330	.810
30	34	.610	
30	35	.580	
30	35	.500	
30	36	.620	.840
30	38	.890	1.130

of the cortex which returns to normal on replacement therapy. Absence of the anterior lobe results similarly in atrophied testes. There is a possibility that adrenalectomy might injure the hypophysis and so destroy the testes.

Daily injections of 1 to 2 cc. of urine of pregnancy containing maturity hormone is capable of stimulating readily the accessory sex organs in the male. After adrenalectomy, 4 or 5 daily injections will double the size of the immature seminal vesicles; the testes in the meantime, however, are rapidly degenerating in spite of the abundance of hypophyseal sex hormone.

5696

Bacteriology of Skin Lesions of Tuberculin Reacting Cattle.

LYMAN L. DAINES AND HAROLD AUSTIN. (Introduced by John F. Kessel.)

From the Department of Bacteriology and Pathology, University of Utah Medical School.

This is a preliminary report on the bacteriology of 189 cases of so-called skin tuberculosis of cattle.

In recent years, as the tuberculosis eradication program is succeeding in reducing the number of typical cases of bovine tuberculosis in cattle, there is a marked relative increase in the number of so-called skin lesions and no-lesion-reactors. In Utah, this has resulted in a large majority of the cattle, which react to the tuberculin test, and which on post-mortem examination, fail to show any lesions, or lesions which are confined to the skin or to the subcutaneous tissue.

More than 90% of the lesions studied in this series came from animals which had given a positive tuberculin reaction. The remainder had not been tested because they were obtained in routine examination of regular abattoir material.

Only one of the 189 skin lesions yielded a typical *Mycobacterium tuberculosis* (bovine type) and this came from an animal which appeared to have generalized bovine tuberculosis. This suggests the occurrence of true bovine tuberculosis skin lesions, but it also indicates the probability that the rather rare positive animal inoculation tests of Day,[1] Watson,[2] Mitchell,[3] and others might have been from similar unusual cases.

Careful search of these skin lesions from tuberculin-reacting cattle revealed the presence, in all of them, of acid-fast rods, as well as one or more forms of acid-fast or non-acid-fast coccoid, diphtheroid, or streptococcoid organisms, or branching filaments. These are all probably different stages or forms of a pleomorphic organism.

All but one of the 189 skin lesions, as well as organs from several no lesion-reactors yielded in culture one or more forms of markedly pleomorphic, facultative acid-fast, Gram positive organisms. Of these, a few strains—the only ones tried up to the present—produce fairly constant results when inoculated into male or pregnant female guinea pigs.

These results are probably more nearly typical of the Preisz-Nocard bacillus *(Corynebacterium ovis)* than of any other known organism.

Morphologically and physiologically these organisms resemble very closely not only *Corynebacterium ovis* but also the Actinomyces described by Walker[4] as the cause of human leprosy and by Walker and Sweeney[5] as the cause of rat leprosy. It is possible

[1] Day, L. Enos, *J. A. V. M. A.*, 1921, **59**, N.S. 12 (6), 769.

[2] Watson (Discussion of paper by Traum, Jacob), *J. A. V. M. A.*, 1929, **74**, N.S. 27 (4), 576.

[3] Mitchell, Chas. A., *J. A. V. M. A.*, 1928, **73**, N.S. 26 (4), 493.

[4] Walker, E. L., *J. Exp. Med.*, 1929, **3**, 167.

[5] Walker, E. L., and Sweeney, M. A., *J. Exp. Med.*, 1930, **4**, 331.

that we have in these organisms a new, undescribed species. The possibility that these pleomorphic forms may be stages in the life cycle of a true, attenuated, mammalian tubercle bacillus must be kept in mind.

Because of the constant occurrence of these forms, practically always in pure culture, in skin lesions of tuberculin-reacting cattle and in the organs of no-lesion-reactors, we are led to believe that they are the cause of nearly all of the skin lesions and of the tuberculin hypersensitiveness of most of the reactors in Utah.

If these organisms are finally shown to be the cause of the usual skin lesions of tuberculin-reacting cattle, their cultural reactions and their effects on guinea pigs may offer a means of definite early diagnosis and probably make unnecessary the slaughter of large numbers of cattle.

Extensive animal inoculations and cultural tests are now under way in an attempt to establish definitely either the positive or the negative rôle of these organisms in the skin lesions of cattle and also in the production of tuberculin sensitization in cattle.

5697

An Improved Gasometric Apparatus for Estimation of Amino Nitrogen.

MAX S. DUNN, B. W. SMART AND K. E. BROWN.

From the Chemical Laboratory, University of California at Los Angeles.

The first apparatus for the quantitative determination of amino nitrogen was devised by Sachsse and Kormann.[1] It consisted essentially of a cylinder for deaminizing the aliphatic amine and a buret for purifying and measuring the nitrogen. Mallet[2] described minor modifications in the form of the apparatus. Brown and Millar[3] proposed an elaborate set-up for carrying out the amino nitrogen determination. Van Slyke[4] devised an apparatus giving satisfactory accuracy yet requiring only relatively simple manipulations. Klein[5] described an all glass deamination chamber to replace the bot-

[1] Sachsse, R., and Kormann, W., *Die landwirthschaftlichen Versuchs-Stationen*, 1874, **17**, 321. *Z. Anal. Chem.*, 1875, **14**, 370.

[2] Mallet, J. W., *U. S. Dept. Agri., Div. of Chem., Bull.*, 1898, **54**, 7.

[3] Brown, H. T., and Millar, J. H., *Trans. Guinness Research Lab.*, 1903, **1**, 18.

[4] Van Slyke, D. D., *J. Biol. Chem.*, 1909-10, **7**, XXXIV. *Ber.*, 1910, **43**, 3170.

[5] Klein, D., *J. Biol. Chem.*, 1911, **10**, 287.

tle and tubes first used by Van Slyke. Later, Van Slyke[6] not only made use of this feature but also added a motor shaking mechanism which, together with the gas buret and Hempel pipet, was mounted in the form familiar today to the many users of his amino nitrogen apparatus. A little later Van Slyke[7] devised his micro apparatus which, except for size, is similar to the larger form. Kupelwieser and Singer[8] and Koch[9] modified the deaminizing chamber to prevent possible losses of nitrogen. Van Slyke[10] published a description of his manometric apparatus and Folley[11] made a new

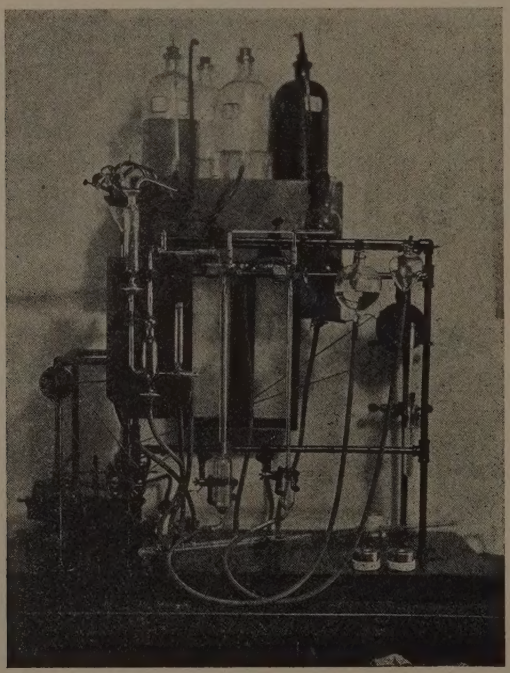

FIG. 1.

Photograph of an improved gasometric apparatus for the estimation of amino nitrogen.

[6] Van Slyke, D. D., *J. Biol. Chem.*, 1912, **12**, 275.

[7] Van Slyke, D. D., *J. Biol. Chem.*, 1913-14, **16**, 121; 1915-16, **23**, 407.

[8] Kupelwieser, E., and Singer, K., *Biochem. Z.*, 1926, **178**, 324, as quoted in *Chem. Abstr.*, 1927, **21**, 1285.

[9] Koch, F. C., *J. Biol. Chem.*, 1929, **84**, 601.

[10] Van Slyke, D. D., *J. Biol. Chem.*, 1929, **83**, 425.

[11] Folley, S. J., *Biochem. J.*, 1930, **24**, 961.

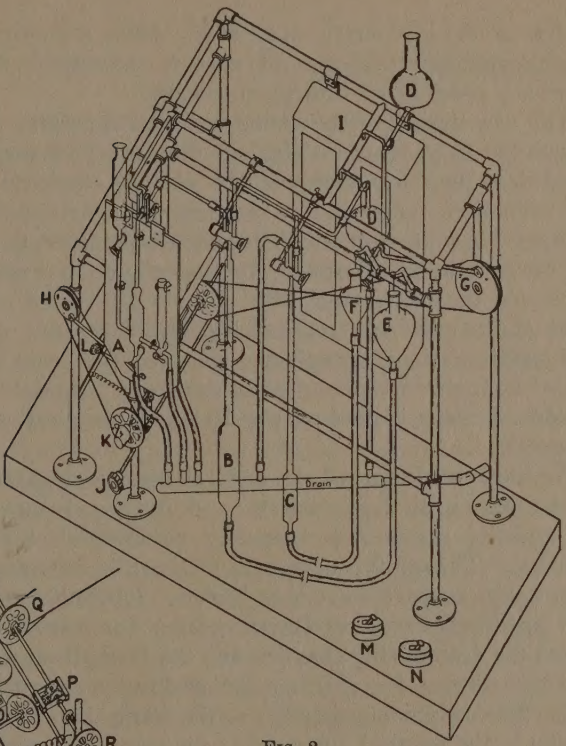

FIG. 2.

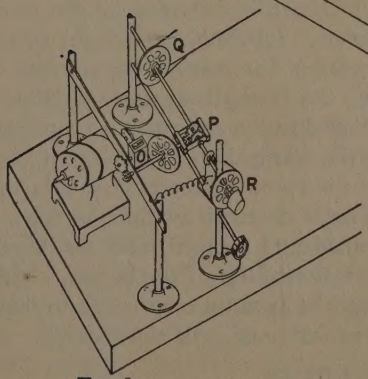

FIG. 3.

FIGURES 2 AND 3.

Drawings to scale of an improved gasometric apparatus for the estimation of amino nitrogen.

A—Board on which deaminizing apparatus is mounted.

B—Macro gas buret, 30 ml. capacity.

C—Micro gas buret, 3 ml. capacity.

D—Hempel pipet with stop cock connected to drain.

E—Leveling bulb for macro buret.

F—Leveling bulb for micro buret.

G—Driving cam for agitating Hempel pipet.

H—Driving cam for agitating deaminizing apparatus.

I—Illuminating chamber with 2 ground glass windows and containing 4, clear glass, 60 Watt bulbs.

J—Control device for gear shift.

K—Shaft upon which the driving gears are mounted and used for shifting the drive from the deaminizing apparatus to the Hempel pipet.

L—Drive for oscillation counter.

M—Control switch for motor.

N—Control switch for illuminating chamber.

O—Oscillation counter.

P—Driving gears.

Q—Pulley for oscillating the Hempel pipet.

R—Pulley for oscillating the deaminizing apparatus.

design of the gasometric apparatus. Here a continuous all glass system eliminates the use of rubber connections through which there is a possibility of nitrogen leakage.

The new design of the amino nitrogen apparatus presented here is believed to be more satisfactory than previous forms. We have recognized the inherent deficiencies and the empirical nature of the determination. Quantitative accuracy in the determination of some amino acids is possible only when the temperature, the rate of shaking the deaminizing chamber, the time allowed for deamination, the value for the blank, and other factors are under precise control. With other amino substances such as glycine, cystine, glycyl peptides, and certain purines, unexplained abnormalities occur and are accentuated by higher temperatures as shown by Schmidt.[12] An investigation of these important physical and chemical problems is in progress.

As shown in Figures I, II, and III the new apparatus consists of a Hempel pipet, 2 gas burets, and the usual Van Slyke macro deaminizing chamber permanently mounted so that breakage is minimal. Though this apparatus has been in constant use for more than a year no parts have been broken. Obvious advantages of the new apparatus are a revolution counter for measuring the rate at which the deaminizing chamber and the Hempel pipet are oscillated, a simple device for shifting the shaking mechanism to oscillate either the deaminizing chamber or the Hempel pipet, a rheostat (not shown in the figures) for varying the speed of the motor, a macro and a micro buret for measuring large or small volumes of gas, a modified Hempel pipet easily drained and cleaned, and an illuminated background to facilitate accurate reading of the burets. Rubber connections are retained throughout because errors due to leakage of the gas through rubber are probably of little significance.

[12] Schmidt, C. L. A., *J. Biol. Chem.*, 1929, **82**, 587.

5698

Immunization Studies with the Virus of Infectious Myxomatosis.

ROY T. FISK AND JOHN F. KESSEL.

From the School of Medicine, University of Southern California, and the United States Bureau of Biological Survey.

Unsuccessful attempts to immunize the domestic rabbit against the virus of infectious myxomatosis by means of various vaccines have been reported by Sanarelli,[1] Moses,[2] and Hobbs.[3] In this study 8 rabbits immunized with phenolized and formalized vaccines prepared from the South American myxoma virus also demonstrated no immunity.

Recently a strain of myxoma virus has been encountered in several rabbitries of Southern California (Kessel, Prouty and Meyer[4]), and this strain has been employed in the accompanying series of vaccination experiments. All of the vaccines were made from virus in the form of fresh sterile blood collected from rabbits showing the symptoms of advanced myxomatosis, and were injected into the experimental animals by the subcutaneous route. Chemically inactivated vaccines were prepared by phenolizing and formalizing 10 and 20% solutions of the myxomatous blood. Heat was also employed as a means of inactivating the virus, and portions of the blood were heated both at 60°C for 30 minutes and at 45°C for 24 hours.

Eight animals vaccinated with heat inactivated virus proved susceptible to subsequent inoculation of living virus. Attempts to immunize rabbits with phenolized and formalized vaccine have yielded more encouraging results, and are summarized in Table I.

In addition to the above 29 control animals, 111 other unvaccinated rabbits have been inoculated with the California strain of virus in other studies in progress. Of these 150 unvaccinated animals, 5, or 3.3%, have shown partial resistance, and 2, or 1.3%, have recovered. No inoculated animals in this series have failed to develop symptoms. Thus a total of 7 unvaccinated animals, or 4.6%, as contrasted with 25.6% of vaccinated animals have exhibited either partial or complete resistance to inoculation.

---

[1] Sanarelli, G., *Centr. Bakt.*, 1898, **30**, 865.

[2] Moses, A., *Mem. Inst. Oswaldo Cruz.*, 1911, **3**, 46.

[3] Hobbs, J. R., *Am. J. Hyg.*, 1928, **8**, 800.

[4] Kessel, J. F., Prouty, C. C., and Meyer, J. W., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 413.

TABLE I.

Showing Number of Animals Immunized with Chemically Inactivated Vaccine.

	39 Vaccinated Rabbits		29 Unvaccinated Rabbits	
	No.	%	No.	%
Partial resistance (*i. e.*, lived 10 days or more after symptoms)	6	15.4	1	3.4
Resisted 2 subsequent I. C. inoculations of virus	2	5	0	0
No symptoms. Complete resistance	1	2.6	0	0
Recovery with subsequent complete resistance	1	2.6		
Total showing partial or complete resistance	10	25.6	1	3.4

The 2 animals that recovered without previous vaccination were of the long-haired, Angora variety. These also developed abscess at the point of inoculation, from which an organism resembling *Pasteurella cuniculicida* was recovered.

Reinoculation experiments with both California and South American strains of virus performed on 2 of the animals recovered from myxomatosis are shown in Table II.

TABLE II.

Strain of Virus	No. 1, Angora Rabbit. Received no vaccine, natural recovery	No. 2, Brown (mixed) Rabbit. Received formalized vaccine prior to inoculation
Californian	Subcutaneous inoculation 14 days after recovery Remained normal Control died 5 days after symptoms	Subcutaneous inoculation 20 days after recovery Remained normal Control died 2 days after symptoms Intravenous inoculation 27 days after recovery Remained normal Control died 1 day after symptoms
South American	Intracutaneous inoculation 28 days after recovery Remained normal Control died 6 days after symptoms Subcutaneous inoculation 37 days after recovery Remained normal Control died 4 days after symptoms	Subcutaneous inoculation 37 days after recovery Remained normal Control died 4 days after symptoms

From this table, it will be seen that a naturally recovered animal, and an animal recovered following vaccination have demonstrated immunity both to the California and South American strains of myxoma virus.

Dr. J. R. Hobbs, of the Harvard University Medical School, by personal communication of May 16, 1931, states that one of his experimental rabbits, naturally recovered from an infection of the South American variety of myxomatosis, has resisted subsequent inoculation with the California strain of virus. Dr. Hobbs' and our results thus indicate that recovery from either South American or Californian types of myxomatosis will render immunity to subsequent inoculations with both strains of virus.

5699

Effect of Vitamin C Diet on Blood Formation in Experimental Scurvy of Guinea Pigs.*

STACY R. METTIER AND WILLIAM B. CHEW.

From the Department of Medicine and Pathology, University of California Medical School, San Francisco.

We recorded[1] that a variable degree of anemia occurred in a large percentage of individuals suffering from vitamin C deficiency. That a reticulocyte response could be induced in such patients by the administration of a diet rich in vitamin C. That the evidence suggested an altered function of the bone marrow, in great measure directly dependent upon the chronic lack of vitamin C. Since anemia, reported by Meyer and McCormick[2] and others is of regular incidence in experimental scurvy of guinea pigs, it seemed desirable to determine the effect of vitamin C-containing foods on blood formation. Accordingly, experiments were made (1) to ascertain the effect of a diet deficient in vitamin C on the bone marrow of the guinea pig; (2) to ascertain the effect of a diet containing vitamin C on the bone marrow administered to animals with manifest scurvy.

Adult guinea pigs weighing between 300 to 550 gm. were used throughout the experiment. The males were segregated from the females and all animals were observed over a preliminary period of one to 3 weeks.

---

* Read before the Section on Medicine of the American Association for the Advancement of Science, Pasadena, California, June 17, 1931.

[1] Mettier, Stacy R., Minot, George R., Townsend, Wilmot C., *J. Am. Med. Assn.*, 1930, **95**, 1089.

[2] Meyer, A. W., and McCormick, L. M., *Stanford University Publications*, 1928, **2**, 199.

The diet to produce scurvy was calculated to be adequate in all food factors with the exception of vitamin C-containing substances.

In a group comprising 10 animals, signs of scurvy began to appear 10 to 15 days after being given the scurvy-producing diet, and death usually occurred in from about 21 to 30 days. Coincident with the appearance of signs of scurvy, there developed an anemia which became progressively more severe. The red blood cells decreased from an average normal of about 5,000,000 per cu. mm. to as low as 2,500,000 per cu. mm. The hemoglobin decreased from an average normal of 12 gm. to as low as 5.5 gm. In all animals the reticulocytes, which had been less than 1% during the control period, appeared in moderately increasing numbers as the anemia became more marked. There was a terminal rise in the reticulocytes of from 5 to 10%.

The bone marrow removed from these animals after death and examined under the microscope showed a marked increased cellularity. There was an almost complete disappearance of fat cells. There was a marked decrease in the number of mature erythrocytes but an increased number of normoblasts. Only an occasional mitotic figure was noted.

In another group of 3 animals with manifest scurvy and definite anemia, 3 cc. of orange juice was fed daily. There followed soon after an augmented rise in the reticulocytes in the peripheral blood in all animals of from 12 to 20% and prompt alleviation of the anemia and disappearance of signs of scurvy.

Sections of bone marrow removed from these animals at the height of the reticulocyte response also showed marked cellularity. In contrast to the bone marrow removed from animals with scurvy before treatment was started, there was a marked increase in the number of mitotic figures and a more active appearance of the bone marrow.

It is concluded from these experiments that the lack of vitamin C from the diet of the guinea pig results in a profound anemia that is accompanied by definite cytological changes in the bone marrow indicative of retarded production of red blood cells.

New York Section.

New York Academy of Medicine, October 21, 1931.

5700

Fresh Raw Aqueous Spleen Extract in Tuberculosis.

GEORGE F. WATSON. (Introduced by F. F. Tisdall.)

From Kitchener, Ontario.

Forty-eight guinea pigs, averaging about 225 gm. were divided into 2 groups of 24 each. On February 24th, 1931, those to be treated were given ¼ cc. of spleen extract subcutaneously in the left groin, and on March 3rd they were given ⅜ cc. On March 5th both groups were inoculated subcutaneously in the right groin with ½ cc. of an emulsion containing a human strain of *Tuberculosis bacilli*, 4 bacilli to a high powered microscopic field. The treated group were then given ⅜ cc. of spleen extract every second day.

TABLE I.

Date	Guinea Pigs Treated by Spleen Extract No. Died	Untreated Guinea Pigs No. Died
March 26	1	3
April 9	0	5
" 16	0	5
" 23	1	2
" 30	0	3
May 5	0	1
" 9	2	2
	Total 4	21

In other words, 83.3% of the treated animals were living as compared with 12.5% of the untreated on May 9th.

Both treated and untreated groups at necropsy showed hypertrophy of the spleen.

In each treated animal at the site of the inoculation a swelling developed which contained caseous material. The untreated animals

did not show this. In other experiments the inguinal glands of untreated animals have suppurated at the site of inoculation and a grey sloughing ulcer resulted. No ulceration has been seen in the treated pigs. Guinea pigs of about 225 gm. in weight are much better for this experiment than the larger pigs, as the smaller animals have practically no spleen and, if given a fairly large dose of *Tuberculosis bacilli,* the controls die early, while, by giving spleen to the other group, one is able to carry on a large percentage for some time.

5701

Changes in Weight of Thyroid Gland of Guinea Pigs Under the Influence of Acid Extract of Anterior Pituitary.*

LEO LOEB AND HILDA FRIEDMAN.

From the Department of Pathology, Washington University School of Medicine.

Loeb, Bassett, and Hilda Friedman[1, 2, 3] have shown that intraperitoneal injections of acid or alkali extracts of the anterior pituitary of cattle cause in the guinea pig a remarkable hypertrophy and hyperplasia of the thyroid gland associated with liquefaction and absorption of colloid. Martin Silberberg[4] has studied the combined effect of KI and this extract on the thyroid gland and W. J. Siebert[5] has determined the effect of various preparations of the anterior pituitary gland on the basal metabolism under various conditions. In our former investigations we used as a criterion of hypertrophy the microscopic changes in the thyroid gland after anterior pituitary injections. The thyroid glands of the injected animals appeared distinctly larger than the normal thyroids.

We sought to determine the changes in weight of the thyroid glands produced by these preparations, in order to obtain quantita-

* These investigations were carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

[1] Loeb, Leo, and Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 860.

[2] Loeb, Leo, and Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 490.

[3] Loeb, Leo, Bassett, R. B., and Friedman, Hilda, PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 209.

[4] Silberberg, Martin, PROC. SOC. EXP. BIOL. AND MED., 1929, **27**, 166; *Krankheitsforschung,* 1930, **8**, 171.

[5] Siebert, W. J., and Smith, R. S., *Am. J. Physiol.,* 1930, **95**, 396.

tive data as to the intensity of the hypertrophy and hyperplasia. We carried out 3 experiments, the results of which are shown in the following table.

TABLE I.

Experiment	No. of Animals		Aver. Initial Weight		Aver. End Wt.		Aver. wt. both thyroids end of experiment	
	Injected	Control	Injected	Control	Injected	Control	Injected	Control
I Daily injection of 1 cc. extract 5 days. Examination on 6th.	8	12	gm. 191	gm. 191	gm. 193	gm. 203.5	mgm. 48	mgm. 23.6
II Daily injection of 1 cc. extract 6 days. Examination on 7th.	12	12	190	192	190.4	198.75	58.7	29.2
III Daily injection of 2 cc. extract 3 days.	3	3	265	264	280	261	72.9	27.6
Weight of individual guinea pigs and of their thyroids in third experiment.								
			265	265			58.6	29.9
			265	260			89.0	29.3
			265	265			71.1	23.5

In the first 2 experiments, the average initial weight of the injected as well as of the control guinea pigs was approximately 191 gm. During the period of treatment (5 days for the first experiment and 6 days for the second), the average weights of the injected animals remained almost unchanged, while the controls gained on the average of 10 and 8 gm. respectively. As a result of the injections the thyroid approximately doubled its weight. In the third experiment in which we had to deal with somewhat heavier guinea pigs, weighing on the average 265 and 264 gm. respectively, the average weight of the thyroid of the injected animal is seen to be approximately 2½ times greater than the weight of the gland of the control guinea pig.

It is of interest to observe that in all 3 experiments, the values for the average weights of the thyroids of the normal guinea pigs are very close, irrespective of the differences in the average weights of the animals. We further notice in the third series, in which double the amount of extract was administered over a shorter period of time, that the average gain in weight of the thyroid glands

of the injected animals was greater than was found in the corresponding guinea pigs of the first and second series which were subjected to half the dose extending over a longer period of time.

5702

A Rapid Method of Conferring Protection to the Peritoneum Against Experimental Peritonitis.*

BERNHARD STEINBERG.

From the Laboratories and the Department of Medical Research of Toledo Hospital, Toledo, Ohio.

Goldblatt and I[1, 2, 3] demonstrated that by immunization with living or heat killed colon bacilli an immunity could be conferred upon dogs against experimental colon bacillus and fecal peritonitis. We found the immunity to be efficient 17 to 18 days after the first immunizing injection and that living organisms produced a greater protection than heat killed bacteria. Confirmatory evidence of an establishment of an immunity was supplied by Herrmann.[4]

The purpose of the work was to determine the shortest time in which an immunity could be conferred upon an animal so that it could survive an experimentally produced peritonitis. To determine the efficacy of different antigens, heat killed colon bacilli (culture 300) and a mixture of heat killed colon bacillus, streptococcus, *B. pyocyaneus, enterococcus, Bacillus mucosus capsulatus* and *B. welchii* was used. The bacteria composing the mixture were isolated from appendices with appendicitis and were of a determined marked virulence to animals. The second variable factor to be determined was the number of injections given to the animal. All the injections were administered intraperitoneally. The experimental peritonitis was produced either by injecting intraperitoneally into dogs the washings of 3 slants of live *B. coli* (300) suspended in 40 cc. of 2½% gum tragacanth or the washings of 3 slants of the bacterial mixture suspended in 40 cc. of a 2½% gum tragacanth.

* This work is a part of a general investigation on "Recovery in Peritonitis" aided in part by a grant by the Committee on Scientific Research of the American Medical Association.

1 Steinberg, B., and Goldblatt, H., *Am. J. Path.*, 1927, **3**, 541.

2 Goldblatt, H., and Steinberg, B., *Arch. Int. Med.*, 1928, **41**, 42.

3 Steinberg, B., and Goldblatt, H., *Arch. Int. Med.*, 1928, **42**, 415.

4 Herrmann, S. F., *Arch. Surg.*, 1929, **18**, 2202.

A series of 4 groups of dogs were immunized with heat killed colon bacilli. One group (12 animals) had 1 injection of 1 billion organisms and on the following day was given *B. coli* peritonitis. Of the 12 animals, 10 died. The second group (10 animals) had 2 injections, the first of 1 billion bacteria and the second on the next day of 2 billion organisms. The day after the second injection, the animals were given colon bacillus peritonitis. Of the 10 dogs, 8 died. The third group (10 animals) had 3 injections of 1, 2 and 3 billion organisms respectively on successive days and *B. coli* peritonitis on the day after the third injection. Of the 10 animals, 9 died. The fourth group (28 animals in 3 different series) had 4 injections of 1, 2, 3 and 4 billion organisms respectively on 4 successive days and *B. coli* peritonitis on the day following the fourth injection. Of the 28 animals, 10 died. There was a survival of 65%. Considering the overwhelming peritonitis which was 3 to 5 times the lethal dose for an average dog (10 to 15 kilograms weight), the results of the 4 injections were considered satisfactory. With each group, normal control dogs were given simultaneously with the protected animals a colon bacillus peritonitis. The control dogs invariably died.

Since the maximum percentage of survivals was obtained with 4 successive immunizing doses with peritonitis on the day following the fourth injection, a similar procedure was employed with the bacterial mixture experiment. Twelve dogs were immunized with 1, 2, 3 and 4 billion bacteria (each organism forming one-sixth of the mixture) on 4 successive days and a colon bacillus peritonitis was given the day following the fourth injection. Of the 12 dogs, 9 died. A percentage survival of 25. All the accompanying normal control dogs with colon bacillus peritonitis died.

To determine the relative number of survivals of the colon bacillus and the bacterial mixture immunized dogs followed by a bacterial mixture peritonitis 2 groups of animals were used. One group of animals (10) was given 4 injections on successive days of heat killed colon bacilli and a bacterial mixture peritonitis on the day following the fourth injection. Of the 10 animals, 7 died. Another group of 9 animals was given 4 injections of a heat killed bacterial mixture on successive days and a bacterial mixture peritonitis on the day following the last injection. Of the 9 dogs, 7 died. The bacterial mixture immunized animals, even following a bacterial mixture peritonitis, showed a smaller percentage of survivals (22%) than the colon bacillus immunized group (30%). The colon bacillus (300) apparently is an excellent antigen in protecting the peri-

toneum against peritonitis. These experiments add to our previously expressed contention[5] that the protective process is not specific in nature. The serum and the peritoneal exudate of the protected animals with peritonitis did not contain humoral antibodies to account for their survival.

The principle of peritoneal protection used in the above experiments was applied to man, prior to abdominal operations requiring resection of bowel. The protecting material consists at present of 50 cc. of physiological salt solution in which are suspended 4 billion colon bacilli (culture 300) and is given in 4 successive daily injections of 5 cc., 10 cc., 15 cc., and 20 cc., respectively.

5703

Effect of Hyperleukocytosis (Hyperleukocytic Pre-Immunity) on Infection.*

BERNHARD STEINBERG.

From the Laboratories and the Department of Medical Research of Toledo Hospital, Toledo, Ohio.

In the previous article[1] it was demonstrated that a single intraperitoneal injection of heat killed *B. coli* may prevent the death of a dog from an otherwise lethal *B. coli* peritoneal infection. The peritonitis was produced the day after the protecting injection. Four successive injections on successive days resulted in the survival of 65% of the animals. No humoral antibodies were found to account for this protection. In order to determine the rôle played by the cellular antibodies, cell counts of the peripheral blood and of the peritoneal exudate and peritoneal bacterial counts were made hourly during the course of a peritoneal infection in normal and protected dogs. Throughout these experiments, peritonitis was produced by the intraperitoneal introduction of 3 billion living *B. coli* suspended in 40 cc. of a 2½% gum tragacanth.

The peripheral leukocytes of normal dogs dropped rapidly and seldom exceeded the count prior to onset of infection. The leukocytes in the peritoneal exudate were polymorphonuclears and were

[5] Steinberg, B., and Snyder, D., *Arch. Path.*, 1929, **8**, 419.

* This work is a part of a general investigation on "Recovery in Peritonitis" aided in part by a grant by the Committee on Scientific Research of the American Medical Association.

[1] Preceding article.

seldom more than 50,000 per cu. mm. The bacteria in the peritoneal exudate were always present in large numbers both free and phagocytosed.

The protected dogs that survived had consistently higher peripheral blood leukocyte counts than the normal animals and the leukocytes rapidly exceeded the count prior to infection. The leukocytes of the peritoneal exudate prior to onset of infection were present in large numbers (232,000 to 546,000 per cu. mm.) and were predominantly polymorphonuclears. After the onset of peritonitis, the number of peritoneal leukocytes dropped considerably and consistently while the total leukocyte counts were higher than in the normal dogs. The bacteria in the peritoneal exudate disappeared rapidly so that in 4 to 5 hours the counts were in thousands instead of many millions as in the normal dogs. The method of bacterial counts employed disclosed the presence of viable organisms whether free or phagocytosed.

The leukocyte counts of the peritoneal exudate of protected dogs that died were consistently lower than those of surviving protected animals but higher than those of normal controls. The cellular response was apparently insufficient to cope with the infection. The bacterial counts, however, in the protected animals that survived or died did not vary. It is assumed that this protective process consists of 2 phases. The first phase comprises the rapid phagocytosis of the bacteria by polymorphonuclears already present at the site of infection. The successful accomplishment of this phagocytosis is the clearing of the first hurdle. The second phase (which is being further investigated) is assumed to transpire at the sites to which the bacteria are transported. The ebb and rise of the peripheral and peritoneal leukocytes probably correspond to migrations of bacteria-laden phagocytes and replacement by new cells.

It is apparent from these experiments that protected dogs respond with a definite peripheral blood leukocytosis upon reinjection of similar living bacteria. The protection may consist of as little as a single injection and the infection may be induced as early as on the following day. The protecting bacterial injection evokes *in situ* a large number of polymorphonuclears. The animal survives largely because of the incidental presence of the polymorphonuclears which phagocytose the living bacteria. The animal is also able to supply rapidly large numbers of new leukocytes to replace those that had been destroyed or that had migrated. This protective process can not be considered either a local or an active immunity in the accepted interpretation of these terms. The animals develop a true

active immunity of a varying degree a number of days following the protecting injections. Because of these factors, the term hyperleukocytic preimmunity is suggested for this process. The question of the specificity of this process will be dealt with in a later article.

Gay and Claypole[2] observed that typhoid immunized animals respond with a peripheral blood hyperleukocytosis upon an intravenous reinjection with living typhoid organisms. They found this hyperleukocytosis to be specific for the microorganism. A typhoid immunized animal did not respond with a hyperleukocytosis to an infection with *Micrococcus aureus*. Gay and Claypole[3] were dealing with a general cellular response in form of an active immunity, since the reinjection was intravenous and was made a sufficient number of days after the first protecting dose to evoke some degree of an active immunity. McWilliams,[4] however, was unable to confirm Gay and Claypole's work neither in regard to the specificity nor to the presence of a hyperleukocytosis. Zinsser and Tsen[5] found a slight leukocytosis in immunized animals but no specificity.

5704

Absorption of Antigens from Body Surfaces.

JOSEPH SIMONS. (Introduced by Lloyd Arnold.)

From the Department of Bacteriology and Preventive Medicine, University of Illinois College of Medicine, and Research Laboratory of the Illinois State Department of Public Health, Chicago.

The appearance of agglutinins in the blood 3 weeks after oral vaccination with typhoid antigen was followed in 85 non-febrile dispensary and ablatory patients. Most patients were from orthopedic surgical clinics. One gram of desiccated ox-bile in gelatin capsules was given with a glass of warm water upon rising in the morning. After 30 minutes the typhoid antigen was administered in a glass of warm water. In one series of 24 subjects, 1 cc. of standard typhoid vaccine (Lilly) was administered. In another series 2 cc. of bacteriophage dissolved *B. typhosus* was administered. The meal was withheld from the subjects for 2 hours after the typhoid

[2] Gay, F. P., and Claypole, E. J., *J. Am. Med. Assn.*, 1913, **60**, 1950.

[3] Gay, F. P., and Claypole, E. J., *Arch. Int. Med.*, 1914, **14**, 662.

[4] McWilliams, H. I., *J. Immun.*, 1916, **1**, 159.

[5] Zinsser, Hans, and Tsen, Edgar, *J. Immun.*, 1917, **2**, 247.

antigen was given by mouth. This was repeated each morning for 3 consecutive doses in each series.

Blood was taken from subjects before experiments were started. All showing the presence of agglutinins against typhoid antigen were eliminated. Three weeks after the oral ingestion of the antigen, blood was again drawn and the agglutination tests performed upon the serum. The accompanying table gives the results.

TABLE I.

Typhoid Vaccine		Bacteriophage Dissolved *B. typhosus*	
Number of Cases	Dilution	Number of Cases	Dilution
4	negative	15	1:40
2	1:20	27	1:80
8	1:40	15	1:160
10	1:80	4	1:320

Bile was given before the oral administration. Arnold[1] explained the influence of bile upon permeability of the intestinal tract in the light of his experimental work. The results reported here seem to show that dissolved *B. typhosus* proteins are more efficient than standard typhoid vaccine for oral immunization.

5705

Susceptibility of Rodents to Gastro-Intestinal Infections.

IRVING KAUFMAN. (Introduced by Lloyd Arnold.)

From the Department of Bacteriology and Preventive Medicine, University of Illinois College of Medicine, and State Department of Public Health, Chicago.

Kisskalt[1] found that the susceptibility of mice to enteritides infections was increased after the administration of saponin. He concluded that this was due to the lowering of surface tension and increasing the spaces between the cells lining the mucosa. We have investigated this problem from the viewpoint advocated by Arnold.[2]

Half-grown mice were given 0.1 cc. of a 20% aqueous solution of saponin by stomach tube. The animals were killed at various time intervals and the bacterial flora and hydrogen-ion concentration of the contents of the stomach, duodenum and jejunum were

[1] Arnold, L., *J. Hygiene*, 1929, **29**, 82.

[1] Kisskalt, K., *Arch. F. Hyg.*, 1929, **101**, 205.

[2] Arnold, L., *J. Hyg.*, 1929, **29**, 82.

determined. The time intervals varied from 15 minutes to 12 hours. There were 180 mice used in the experiment recorded in the accompanying graph. The graph illustrates the changes produced within a 6-hour period of time after the introduction of saponin. There was little variation found in the 20 mice used for each time interval experiment. There is a sudden change in the hydrogen-ion concentration and the bacterial flora following intragastric application of saponin.

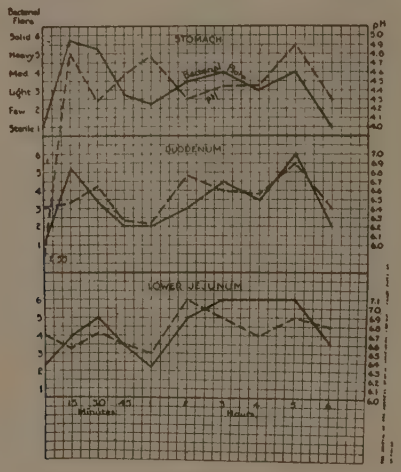

FIG. 1.

Hydrogen-ion concentration and relative density of bacterial flora in stomach, duodenum and lower jejunum of mice after oral administration of saponin.

Ordinate represents time at indicated intervals after ingestion of saponin.

Abscissa on left margin represents relative density of bacterial flora. Solid line illustrates the bacterial flora within the various segments in relation to time as indicated in ordinate.

Abscissa on right margin represents H-ion concentration of the contents of segments of alimentary tract after saponin ingestion.

Organ cultures after the introduction of saponin in the stomach accompanied by a dose of *B. enteritides* show a widespread distribution of this organism within the body of the animal. When the same dose of bacteria is introduced without saponin the organs were found to be sterile after the same period of time. The increased susceptibility observed by Kisskalt,[1] seems to be intimately associated with certain demonstrable changes in the bacterial flora and acid-base equilibrium of the stomach and small intestine. The changes within the intragastric and intestinal contents have been shown to take place as a result of certain alterations in environment of the animal (Arnold[2]). Saponin causes a sudden alteration in

the acid-base equilibrium and in the bacterial flora of the contents of the stomach and small intestine.

5706

Changes in Respirations Produced by Surgical Operations.

HERBERT A. CARLSON. (Introduced by O. H. Wangensteen.)

From the Department of Surgery, University of Minnesota.

Reduction of vital capacity and elevation and decreased excursions of the diaphragm are significant alterations of the respiratory function known to follow certain surgical operations, notably those on the abdomen. The hypoventilation[1] resulting from the postoperative embarrassment of respiration has been regarded as a factor leading to the development of atelectasis and pneumonia.

To obtain data regarding changes of the separate components of respiration, pneumograph tracings were made before and after operation on a series of hospital patients. The method employed was a modification of that described by Greisheimer[2] in which rubber bags (from sphygmomanometers) were fitted into covers of sufficient length to encircle the body of the patient. One tube was attached to a bulb for the inflation of the bag; the other tube was connected by pressure tubing to a water manometer. One bag was placed about the thorax as high as possible, another around the abdomen below the costal margins. In this manner thoracic (intercostal) and abdominal (diaphragmatic) respirations were recorded synchronously on a smoked surface. In order to make conditions of the experiment uniform, all tracings were taken with the patient in the supine position and with such a pressure in the inflated bag that at expiration the pointer was 2 cm. above the base line. Observations were made on 58 patients, having operations in various anatomical regions as follows: upper abdomen, 19; lower abdomen, 21; thyroid, 9; thorax 5 (4 thoracoplasties); perineum or extremities, 4. The observations reported here are based on more than 200 tracings.

Results. In the following tables, the maximum, minimum, mean and median changes in rate of respiration and depth of thoracic and

[1] Müller, G. P., Overholt, R. H., Pendergrass, E. P., *Arch. Surg.*, 1929, **19**, 1322.

[2] Greisheimer, E., *Minn. Med.*, 1925, **8**, 387.

abdominal breathing are recorded in terms of per cent of the original pre-operative figures. In indicating postoperative days, the day of operation is referred to as the first day. The number of readings on which the percentages are based are indicated for each day in the tables.

TABLE I. *Upper Abdominal Operations.* (19)

				Rate of Respiration					
Days	1	2	3	4	5	6	7	8-10	11+
No. Readings	10	8	5	5	8	3	4	7	8
	%	%	%	%	%	%	%	%	%
Maximum	206	193	158	213	207	140	147	177	129
Minimum	103	85	118	105	105	96	109	100	95
Mean	134	129	134	132	139	112	124	132	112
Median	129	134	128	109	142	100	121	126	114
				Thoracic Excursion					
Days	1	2	3	4	5	6	7	8-10	11+
No. Readings	10	8	5	5	8	3	4	7	8
	%	%	%	%	%	%	%	%	%
Maximum	650	700	333	200	166	183	157	146	133
Minimum	44	122	80	40	60	100	80	77	47
Mean	162	223	147	114	107	154	113	112	82
Median	97	160	106	106	103	181	109	120	83
				Abdominal Excursion					
Days	1	2	3	4	5	6	7	8-10	11+
No. Readings	10	7	5	5	7	3	4	7	8
	%	%	%	%	%	%	%	%	%
Maximum	82	50	75	100	58	150	333	77	100
Minimum	0	1	20	14	16	42	44	42	42
Mean	29	23	47	59	35	78	133	57	77
Median	33	22	50	66	30	42	78	55	77

TABLE II. *Lower Abdominal Operations.* (21)

				Rate of Respiration				
Days	1	2	3	4	5	6	7	8+
No. Readings	9	14	3	5	4	4	4	16
	%	%	%	%	%	%	%	%
Maximum	211	164	136	148	121	140	130	166
Minimum	95	58	71	100	95	105	90	60
Mean	133	126	101	122	114	123	105	120
Median	118	130	95	120	120	123	100	113
				Thoracic Excursion				
Days	1	2	3	4	5	6	7	8+
No. Readings	9	14	3	5	4	4	4	16
	%	%	%	%	%	%	%	%
Maximum	175	350	154	400	140	183	233	270
Minimum	77	30	39	40	64	40	53	35
Mean	122	157	88	187	102	119	141	114
Median	133	163	72	170	104	126	139	96
				Abdominal Excursion				
Days	1	2	3	4	5	6	7	8+
No. Readings	9	13	3	5	4	4	4	16
	%	%	%	%	%	%	%	%
Maximum	225	125	50	133	175	116	133	209
Minimum	0	0	0	10	0	80	0	0
Mean	65	53	21	67	58	101	82	114
Median	45	50	15	63	29	104	98	119

The tables show that an increased rate of breathing occurs following abdominal operations. The average depth of thoracic breathing increases and that of abdominal breathing decreases during the first few days after both upper and lower abdominal operations. It will be observed, however, that in the case of upper abdominal operations, the reduction in abdominal excursion is greater than in the case of lower abdominal procedures.

The observations in other anatomical regions are probably too few for definite conclusions but it is noteworthy that no apparent reduction of the amplitude of abdominal excursions occurs. Following thyroidectomies there was some decrease in depth of thoracic breathing without significant alteration of the abdominal excursions. Following thoracic operations, there was evidently an increased abdominal breathing with a variable effect on thoracic breathing. Following operations on the extremities or perineum, significant changes did not occur.

The increased rate of respiration following abdominal operations is probably due chiefly to the reduction of vital capacity but other factors such as fever, pain, and anxiety, may also accelerate the rate. The decrease in respiratory rate occurring in some instances may be the result of the administration of morphine.

There are a number of factors believed to contribute to the decrease of diaphragmatic breathing, such as pain, reflex splinting of the diaphragm and abdominal muscles, alterations of intra-abdominal pressure, abdominal distention and the effect of sedatives. Experiments being carried on indicate that the greatest inhibition of abdominal breathing occurs after the effects of the anesthetic (spinal) have disappeared, *i. e.*, when the patient is having pain. This observation suggests that voluntary or involuntary splinting of the diaphragm and abdominal muscles is most important of the several factors leading to decreased diaphragmatic breathing.

An increase in the amplitude of intercostal breathing often occurs and apparently serves to compensate for the decreased function of the diaphragm.

This study by means of respiration tracings has been supplemented by a series of vital capacity studies. A measure of the patient's ability to blow up a column of water in a glass tube was also made. Both of these functions are greatly diminished postoperatively and will be discussed more fully at a later date.

Conclusions. These observations provide additional evidence that there is a post-operative embarrassment of respiration follow-

ing abdominal operations and that it is the diaphragmatic component that is primarily affected.

Inasmuch as post-operative pulmonary complications usually occur in the lower lobes and most commonly follow operations on the abdomen, it is probably more than a coincidence that abdominal operations result in decreased diaphragmatic excursions with the attendant diminution of ventilation of the lower lobes of the lungs.

5707

Fermentation of "Levan" by Pneumococci.

JOSÉ ZOZAYA.

From the Mulford Biological Laboratories, Sharpe & Dohme, Glenolden, Pa.

The studies of Hiss[1] show that inulin is fermented by the pneumococcus organism and that this fermentation can be used as a method of differential diagnosis between the Pneumococcus group and the Streptococcus. Bergey[2] mentions a *Streptococcus inulinaceus* Orla-Jensen, which also ferments inulin. A differentiation between these two organisms can be made by using other sugars. Among fungi, the presence of an inulase appears to be widespread, and inulin decomposition has been observed both among higher and lower types. Bourguelot[3] found inulase in *Aspergillus niger;* Grüss[4] in *Ustilago maydis;* Dean[5] in *Aspergillus niger* and a Penicillium; Frou[6] in Morchella; Wehmer[7] in a species of Mucor; Hanzawa[8] in *Rhizopus delemar;* and Castellani and Taylor[9] in *Monilia macedoniensis.*

In view of the work of Harrison, Tarr and Hibbert[10] and of Hibbert, Tipson and Brauns[11] on the constitution of levan and its relation to inulin, it seemed of interest to investigate the fermenta-

1 Hiss, P. H., *J. Exp. Med.*, 1905, **7**, 547.

2 Bergey's Manual of Determinative Bacteriology, 1930.

3 Bourgelot, E., *Comptes rend.*, 1893, **116**, 1143.

4 Grüss, J., *Berichte d. deutsch. botan. Gesellsch.*, 1902, **20**, 213.

5 Dean, A. L., *Botanical Gazette*, 1903, **35**, 24.

6 Frou, Cr., *Comptes rend.*, 1905, **140**, 1187.

7 Wehmer, C., *Lafar's Handbuch der techn. Mykologie*, 1905, **4**, 527.

8 Hanzawa, J., *Mykolog. Zentrbl.*, 1912, **1**, 76.

9 Castellani, A., and Taylor, F. E., *Biochem. J.*, 1922, **16**, 655.

10 Harrison, F. C., Tarr, H. L. A., and Hibbert, H., *Can. J. Research*, 1930, **3**, 449.

11 Hibbert, H., Tipson, R. S., and Brauns, F., *Can. J. Research*, 1931, **4**, 221.

tion reactions of levan obtained from *B. mesentericus* and from *B. subtilis* as well as of dextrans obtained from two different strains of *Leuconostoc mesenteroides.**

The method used was as follows: Sugar-free bouillon plus 10% normal horse-serum and 1% Andrades' indicator. The carbohydrate was added after filtration through a Berkefeld candle in order to make a final concentration of 1%. A control of the horse-serum, sugar-free broth, without carbohydrate, and each of the sugar media was seeded from an 18-hour blood-agar, slant culture of virulent "S" pneumococcus Types I and II, and also from an "R" pneumococcus of the same type. The cultures were incubated at 37°C. and reactivity observed after periods of 24 hours and 4 days respectively. Readings were checked against the control, which latter exhibited no reactivity as denoted by its inability to produce a color change in the medium. The results are shown in Table I.

TABLE I.

Fermentation Tests with Pneumococci and the Levan, Dextran and Inulin Polysaccharides.

		Pn I (37)	Pn II (1599)	Pn I "R" (379)	Pn II "R" 1599 I_9	Pn II "R" (R.I.)
Levan	24 hr.	±	±	—	—	—
B. mesentericus	4 days	+	+	+	+	+
Levan	24 hr.	—	—	—	—	—
B. subtilis	4 days	+	+	±	±	±
Dextran	24 hr.	—	—	—	—	—
(Leuconostoc Mesenteroides A^3)	4 days	—	—	—	—	—
Dextran	24 hr.	—	—	—	—	—
(Leuconostoc Mesenteroides A^6)	4 days	—	—	—	—	—
Inulin	24 hr.	++	—	++	—	—
	4 days	++	++	++	±	+
Control	24 hr.	—	—	—	—	—
	4 days	—	—	—	—	—

— No acid production; ± very slight acid; + distinctly acid, and ++ strongly acid.

The results show that levan but not dextran undergoes fermentation, although this change is less pronounced than with inulin. This difference may possibly be explained as due either to a diminished action of the "inulase" of the pneumococci on levan or to the lower activity of an enzyme capable of fermeting levan. The acidity in both cases seems to be mainly due to lactic acid obtained from the fructose, the latter formed by hydrolysis of the levan.

* We wish to thank Prof. Hibbert for his generosity in supplying these polysaccharides and correcting the manuscript.

Haworth[12] showed that inulin is a polymerized anhydro-fructo furanose, the linkages being at positions 1 and 2 of the fructose chain, while levan is formed by linkages at positions 2 and 6, as shown by Hibbert and co-workers. The structure of dextrans is unknown but is being actively investigated by the latter authors.

In view of the present results, is it to be assumed that pneumococci possess 2 different enzymes, one specific for inulin and another for levan, or that the same enzyme is capable of splitting both of these polysaccharides, which while polymerized fructo-furanoses yet differ markedly in structure? It seems possible, in the light of the suggestive and interesting researches of Quastel,[13] who assumes the possibility of one cell possessing at least 56 "hydrogen transportases" (Thunberg's terminology), that different enzymes are actually present.

Much further work is needed to settle this fundamental point regarding enzymic behavior of microorganisms on various chemical substances. The various enzymes apparently exert a very specific and selective action with respect to the chemical structure.

5708

Seasonal Changes in Frog's Muscles and Their Bearing on the Clasping Reflex.

ERNST GELLHORN AND DAVID NORTHUP.

From the Department of Animal Biology, University of Oregon, Eugene.

Sommerkamp[1] showed that different striated muscles reacted differently to contracture producing substances such as acetyl choline, nicotine, etc., and explained his results by the assumption that certain muscle fibers (tonus fibers) reacting specifically to contracture producing substances are unequally distributed in different muscles. It is apparent that these investigations have great importance in the study of tonus. Therefore experiments were carried out in which the isometric tension of the flexors of the forelimb was determined in male and female frogs *(Rana esculenta)*. Acetyl choline (1:10,000), KSCN (0.113M) and veratrin (1:1,000,000) were used during and after breeding season. The results are sum-

[12] Haworth, W. N., and Learner, A., *J. Chem. Soc.*, 1928, 619.

[13] Quastel, J. H., *Trans. Faraday Soc.*, 1930, **26**, 115.

[1] Sommerkamp, H., *Archiv. Exp. Path. und Pharmakol.*, 1927, **128**, 99.

marized in Tables I and II. While the isometric tension set up by KSCN expressed in percentage of the tetanus tension produced by a just maximal current is practically identical in males and females the experiments with acetyl choline show that the muscles of male frogs react specifically. The tension in males averages 13% and in females 3.2%. It is interesting that several weeks after breeding season an intermediate value averaging 5.6% is obtained from males.

TABLE I.

Contracture tension in % of tetanic tension with maximal current.

No. of Exp.	KSCN 0.113M	Acetyl choline 1:10,000	
	%	%	
9	10	—	
7	—	13.8	Clasping males. During breeding season.
8	9.8	—	
8	—	12.9	Non-clasping males. During breeding season.
10	—	5.6	" " After breeding season.
19	—	3.2	
13	8.2	—	Females. After breeding season.

The isometric curve of flexors of the forearm which were poisoned with veratrin sulphate revealed the specific reaction of the male muscles during breeding season in a still more striking manner. When stimulated with a just maximal faradic current the tension of the slow contracture beginning immediately after the twitch was regularly greater than the twitch tension in males during breeding season; in females the contracture tension was the lower. In these experiments again an intermediate reaction was observed in males several weeks after breeding season. The specific reactions are restricted to the flexors of the forearm since other muscles like *M. gastrocnemius* even in the breeding season react alike in males and females.

TABLE II.

Secondary veratrin tension expressed in % of that of the primary twitch tension with maximal current.

No. of Exp.	%	
9	147	Males during breeding season
8	97	Males after breeding season
15	78	Females

The mechanism of these reactions is the objective of further studies.

5709

Effect Upon the Kidney of Feeding Large Amounts of Amino Acids for a Short Period of Time.

HOWARD H. BEARD AND ROBERT A. MOORE.

From the Department of Biochemistry and Institute of Pathology, Western Reserve University, Cleveland, Ohio.

It has been claimed by many investigators that high protein diets produce renal injury.[1] Newburgh and Curtis[2] have shown that the type and degree of injury is dependent on the nature and amount of the protein and the duration of feeding. In an earlier study Newburgh and Marsh[3] had definite renal injury in 24 to 48 hours following the intravenous injection of certain amino acids. During the course of an investigation on the metabolism of creatine[4] a series of animals became available for histological study of the effects of large quantities of ingested amino acids for short periods of time.

A total of 81 rats, weighing between 40 and 60 gm. have been examined, 48 were fed amino acids, 10 other nitrogenous substances, and 6 were normal. All food except water was removed from the cage at night and the next morning 1 gm. of the substance to be fed was mixed with Sherman's diet B (consisting of whole wheat flour two-thirds, whole milk powder one-third, NaCl and $CaCO_3$, each, to the extent of 1% of the wheat flour) and placed in the cage. Twenty-four hours later the animals were sacrificed by a blow on the head. The kidneys were weighed, sectioned saggitally, fixed in Zenker's solution, embedded in paraffin and stained with hematoxylin and eosin. Two animals were fed phenylalanine, 5 glutamic acid, 6 tyrosine, 5 glycine, 3 alanine, 11 arginine, 3 aspartic acid, 1 leucin, 6 cystine, and 3 histidine. In the second group 7 were fed creatine and 3 glycocyamine.

The absolute kidney weight and the kidney weight body weight ratio show no differences in the several groups. Sections were examined with concealed key numbers and later with knowledge of the source of each specimen. In neither case could any deviation from normal in the structure of the glomerulus or tubules be demonstrated.

We conclude that the ingestion of 1 gm. of certain amino acids, 24 hours previous to examination, produces no morphological change in the kidney of the young white rat.

---

[1] Editorial, *J. Nutrition*, 1929, **1**, 271.

[2] Newburgh, L. H., and Curtis, A. C., *Arch. Int. Med.*, 1928, **42**, 800.

[3] Newburgh, L. H., and Marsh, P. L., *Arch. Int. Med.*, 1925, **36**, 682.

[4] Beard, H. H., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 454.

5710

Oxygen Consumption Without Carbon Dioxide Production in Acidified Tissues.

W. R. AMBERSON, P. B. ARMSTRONG AND W. S. ROOT.

From the Marine Biological Laboratory, Woods Hole, Mass.

In the course of measurements of the respiration of several vertebrate tissues by the tonometric method described by Amberson[1] we detected a very persistent oxygen consumption, not associated with carbon dioxide production, which appears after acidification. This effect was first observed in the eggs of *Fundulus heteroclitus*. It has since been detected in frog muscle. In the lateral line nerve of the dogfish a persistent oxygen consumption after acidification has also been observed, but in this tissue carbon dioxide continues to be produced in small quantities, amounting to about 30% of the oxygen which is consumed.

This effect is not at once evident after acidification, but can be demonstrated in about one hour in the Fundulus eggs and embryos. The immediate effect of the addition of acid to this material is a burst of respiratory activity, together with the liberation of combined carbon dioxide. This phase is over in about half an hour. If the dissolved carbon dioxide be now removed by aeration, it can be shown that in succeeding periods of time, up to five days, oxygen disappears without any associated carbon dioxide production. The magnitude of this persistent oxygen consumption appears to be related to the stage of development, being greater in embryos of 8 to 12 days than in fresh eggs. In embryos of this age the oxygen consumption continues at a level of from 5 to 10 cu. mm. per gm. of fresh weight per hour, or approximately 7 to 14% of the previous respiratory rate. The rate of oxygen consumption is usually greater on the second day after acidification. This oxygen consumption persists even after neutralization of the acid. It is not affected by 0.03M potassium cyanide and is thermostable, continuing in eggs which have been boiled as much as one hour.

Acidification of the gastrocnemius muscle of the frog results in the liberation of carbon dioxide from the carbonates present and a depression of the rate of respiration which falls to about 5 to 10% of the resting value at the end of 2 hours. (Fig. 1.) The rate of carbon dioxide production slowly decreases and disappears between 2 and 24 hours after acidification. Muscles differ considerably in

[1] Amberson, W. R., *Biol. Bull.*, 1928, **55**, 79.

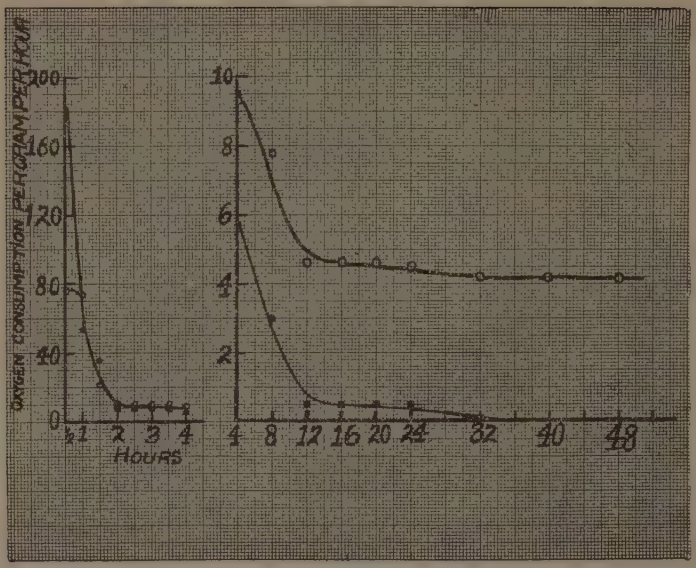

FIG. 1.

The effect of acidification upon gaseous exchanges of frog gastrocnemius muscle.
Ordinate—cu. mm. oxygen consumption per gm. of fresh weight per hour.
Abscissa—time in hours after acidification.
o oxygen consumption.
• carbon dioxide production.

the time at which carbon dioxide production ceases, but in all the terminal rate of oxygen consumption is surprisingly constant at 4 to 6 cu. mm. per gm. of fresh weight per hour for at least 76 hours. Boiling for one hour does not alter the character of the effect.

5711

Effect of Freezing on Microorganisms in Various Menstra.

F. W. TANNER AND G. I. WALLACE.

From the Department of Bacteriology, University of Illinois, Urbana.

We showed[1] that prolonged action of freezing temperatures destroyed many yeasts and bacteria although with some species absolute sterility of the suspensions was not attained even after freezing at —15°C for 160 weeks. These investigations have been continued

[1] Tanner, F. W., and Williamson, B. W., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 377.

to determine the behavior of microorganisms in a large number of commercially frozen fruits and vegetables. Although the numbers of viable microorganisms have shown considerable reduction, there was no sterility even after storage at —16°C for about 2 years. Some 18 pure cultures of various microorganisms have been maintained in the frozen condition in 16 different menstra of varying hydrogen ion, salt and sugar concentrations as well as several fruit juices. To date the suspensions have been frozen for 19 months. In each case there has been a distinct decrease in the numbers of viable cells.

Escherichia coli, a short non-spore-forming rod from cherries, a similar organism from strawberries and the molds showed the greatest decrease in numbers. *Bacillus subtilis* and the spore former from strawberries showed the greatest resistance to prolonged freezing. The presence of acid seemed to have considerable effect on the destruction of bacteria by freezing. Microorganisms suspended in cherry juice and strawberry juice with a pH of about 3.8 and plain broth with a pH of 4.0 and 5.5 showed a greater decrease than in other menstra. While this same effect was noticeable with the yeasts and molds, it was not as pronounced as with the bacteria. Suspensions in strawberry and cherry juice, either sweetened or unsweetened, behaved very much like those in plain broth. Reactions on the alkaline side of neutrality were not as harmful as those on the acid side. High concentrations of sodium chloride seemed to cause a more rapid decrease in numbers of living cells especially in the higher concentrations used (6%). Suspensions frozen in distilled water and various concentrations of sugar showed very slow decrease. The molds died rather quickly but, after 16 months, there were still a very few living cells showing that storage at below freezing does not destroy all of the cells. Non-spore-forming bacteria, especially when frozen in an acid medium, were completely destroyed in from 5 to 10 months.

Alternate freezing and thawing was no more destructive to microorganisms than continuous freezing.

An attempt was also made to determine whether different degrees of cold might influence the death rate of pure cultures of microorganisms in distilled water and cherry juice. The suspensions were held at —16°C, —40°C, and —79°C. When the microorganisms were suspended in distilled water, there were no noticeable differences in death rates at the different temperatures. *Escherichia coli* in cherry juice showed slightly greater longevity at —79°C than at —40°C, and —16°C.

Experiments with *Clostridium botulinum* in several vegetables and a few fruits showed that spores of the organism survived freezing at —16°C for 14 months. The toxin also showed no decrease in toxicity when stored at —79°C for 2 months or at —16°C for 14 months. Vegetables to which detoxified spores were added before freezing at —14°C for 14 months, became toxic in from 3 to 6 days when allowed to thaw and stand at room temperature. This indicates that frozen fruits and vegetables must be considered as perishable products. They differ in this respect from sterilized canned foods. With frozen fruits, despite a pH which ordinarily prevents toxin formation by *Cl. botulinum*, toxin was formed in a few instances. Its formation may have been made possible by development of molds which altered the pH sufficiently to permit development of *Cl. botulinum*. The appearance of the foods in most of these cases would have indicated that they were abnormal due to incipient decomposition. Meyer and Gunnison[2] reported a similar situation with canned bartlett pears.

Experiments are in progress on the longevity of members of the colon-typhoid group in frozen cherries. When suspended in the clear juice and held at —14°C, the organisms died out in 2 weeks. However, when held at —16°C in the presence of both cherries and juice they remained viable for 5 months as proven by bacteriological and serological identification. However, after this time it has been possible to isolate organisms which culturally are like the organisms with which the experiment was started but they seem to have lost their ability to respond to agglutinin.

5712

Bone Marrow Volume in Rabbits.*

ROBERT N. NYE.

From the Thorndike Memorial Laboratory, Second and Fourth (Harvard) Medical Services, Boston City Hospital.

Only a few papers pertaining to bone marrow volume can be found in medical literature. Töppich[1] determined the bone marrow volume

[2] Meyer, K. F., and Gunnison, J. B., *J. Inf. Dis.*, 1929, **45**, 147.

* Aided in part by a grant from the William W. Wellington Fund, Harvard Medical School.

[1] Töppich, G., *Arch. f. Anat.*, 1914, 9.

or mass of 2 newly born infants and found that it was equivalent to approximately 2.3% of the body weight. He employed a method previously described by Wetzel,[2] in which the marrow volume was calculated from the dried weight of the skeleton, the total gross volume of the skeleton and the specific gravity of bone. As infant bone marrow consists practically entirely of red marrow, this figure represented the functioning mass. Wetzel[3] later reported a red marrow volume of 1419 cc. in the skeleton of a 20-year-old man. Mechanik[4] determined the marrow volume in 13 adult cadavers, using a method based upon the weights of the bones before and after maceration. The marrow mass varied from 1600 to 3700 gm., with an average figure of 2600 gm. He believed the red and yellow portions of the marrow were about equal, giving an average value of about 1300 gm. to the active portion, equivalent to approximately 2.3% of the body weight.

Since some idea of the bone marrow volume in rabbits was required in certain experimental work and since no figures could be found other than those relating to human marrow, 2 normal rabbits were killed and marrow volume determinations made with the results indicated below. The method used was essentially that employed by Töppich.[1]

The rabbits were killed by the intravenous injection of ether. The soft parts were cut away as cleanly as possible and the skeletons were covered with water and placed in an incubator (37.5°C). The water was changed twice a day. As many tendons and some muscle tissue still remained after 6 days' maceration, the skeletons were covered with veal infusion broth and 5.0 cc. of an actively growing culture of *B. histolyticus* were added. After 6 days' incubation the bones were completely cleaned of all tendons and muscle. Daily tests of the reaction showed no acid production. The bones were washed in running water for about 8 hours each day and covered with water and placed in the incubator for the balance of the time for 5 days and were then washed continuously in running water for 3 days. They were dried at 47°C to constant weight. After immersion in sulphuric ether for 24 hours, the bones were removed and were cleaned of all loose cartilage and fatty and waxy substances. They were immersed in fresh sulphuric ether for 24 hours and this was repeated 6 times. The bones, after each treatment with ether, were placed in a vacuum to draw out the old ether

[2] Wetzel, G., *Arch. f. Entwicklungsmech.*, 1910, **30**, 507.

[3] Wetzel, G., *Anat. Anz.*, 1920-21, **53**, 82.

[4] Mechanik, N., *Zeitschr. f. Anat. u. Entwicklungsgisch.*, 1926, **79**, 58.

and were again placed in a vacuum, after the fresh ether had been added, to ensure the filling of the marrow cavities. They were dried at 108°C to constant weight and the total weight of each skeleton noted. A boiling hot 4% watery solution of agar agar was poured over the bones and boiling was continued under reduced pressure for about 10 minutes. Keeping the agar agar solution well above the temperature of solidification, each bone was picked from the solution, rapidly rinsed in hot water (±60°C) and immediately plunged in iced water. After the surface water had dried, each bone was carefully examined to be sure that no spaces, except the marrow cavities, were filled by the agar agar. This applied particularly to the bones of the skull. In addition, obvious empty marrow spaces (those of the bodies of the vertebrae which opened into the spinal canal) were filled with vaseline. The lip of a tall glass cylinder was melted and drawn out and downward to a small tip. The cylinder was filled to overflowing with freshly distilled water and, after it had emptied to the level of the lip, all the bones of one skeleton were added one by one. Care was taken to be sure that no air bubbles or pockets remained on or in the bones. The weight of the water displaced was measured and the total gross volume of the skeleton calculated by applying a correction for temperature. The bones were again dried on the surface and the displacement redetermined.

For ascertaining the specific gravity of rabbit bone, a femur and humerus were each cut with a saw into three pieces of approximately equal length. These were cleaned, defatted and dried exactly the same as the skeletons. The weights in air and in distilled water were measured and the specific gravities calculated, applying proper temperature corrections. Previous to weighing in water the pieces were immersed in distilled water and placed in a vacuum to ensure the absence of air bubbles and pockets.

To estimate the relative amounts of red and yellow marrow, all the long bones of the extremities of another rabbit were split lengthwise and examined grossly.

The values for the specific gravity of rabbit bone, as determined by comparing the weights in air and in water of the 6 portions of femur and humerus, are given in Table I. The densities of the shafts are somewhat higher than those of the ends. The average specific gravity (2.302) is somewhat higher than the value (2.145) for human bone given by Wetzel.[2]

The measurements used in calculating the marrow volumes of the two rabbits are given in Table II. Knowing the weight of the dried bones, the volume of the bony substance is obtained by divid-

TABLE I.
Determination of Specific Gravity of Rabbit Bone.

Determination	Femur			Humerus		
	Shaft	Proximal End	Distal End	Shaft	Proximal End	Distal End
No. 1	2.430	2.288	2.187	2.360	2.183	2.268
No. 2	2.436	2.305	2.034*	2.425	2.257	2.291
Average	2.433	2.297	2.187	2.393	2.220	2.280

Average of all determinations = 2.302. * Omitted (probably an air bubble).

TABLE II.
Calculation of Rabbit Bone Marrow Volume.

	Rabbit No. 557 ♂	Rabbit No. 558 ♀
Body weight	2600 gm.	2115 gm.
Bone weight	89.3 gm.	74.3 gm.
Skeleton displacement	105.9 cc.	74.5 cc.
Bone volume	38.8 cc.	32.3 cc.
Marrow volume (weight)	67.1 cc. (gm.)	42.2 cc. (gm.)
$\frac{\text{Marrow weight}}{\text{Body weight}} \times 100$	2.59	2.00

ing the figure for the weight by the figure for the specific gravity of bone. The marrow volume is then obtained by subtracting the volume of the bony substance from the gross volume of the bones, as measured by displacement. This figure can be considered the marrow weight, for Mechanik[4] found that the specific gravities of both varieties of marrow were practically 1.000 (red slightly more, yellow slightly less). In terms of body weight the total marrow masses were 2.6 and 2.0%, respectively, an average of 2.3%. This average is identical with that of Töppich[1] for newly born infants and exactly one-half the value given by Mechanik[4] for adults.

Gross examination of longitudinal sections of the long bones of both extremities indicated that, although fatty marrow did occur, particularly in the radii and ulnae, there was relatively much more red marrow than in the long bones of normal humans. Accepting Mechanik's[4] statement that the red and yellow marrows are approximately equal in humans, it would seem reasonable to estimate that the marrow in the rabbit consisted of about 75% red marrow. This value would be equivalent to 1.7% in terms of body weight. It is appreciably lower than the figures given for infants[1] and adults,[4] which are identical (2.3%), but would be expected since the blood volume of rabbits in terms of body weight is considerably lower than that of humans.[5]

[5] Erlanger, J., *Physiol. Rev.*, 1921, **1**, 177.

5713

Thymus Extirpation in the Laying Hen.

ALAN W. GREENWOOD AND J. S. S. BLYTH. (Introduced by Oscar Riddle.)

From the Department of Genetics, University of Edinburgh.

In an endeavor to test the claims of Soli[1] that extirpation of the thymus gland of the laying fowl leads to the total temporary suppression of shells on eggs laid subsequently, this gland was removed from 3 one-year-old, actively laying, pure-bred Brown Leghorn hens which had been carefully trap-nested since the onset of the laying period. All 3 birds laid in the morning of the day their thymus glands were removed. Recovery was uneventful but the immediate effect was a temporary cessation of egg laying. One bird (No. 29) recommenced laying 10 days later while the other 2 (Nos. 39 and 94) started again 15 days after the operation. All laid regularly thereafter until killed 8 to 9 weeks later.

The eggs laid in the postoperative period were, with one exception, normal in respect to size and shell thickness. The one exception was provided by No. 29 which laid a small egg 61 days after the date of operation. Since this bird produced 2 small eggs within a few weeks immediately preceding the removal of the thymus the small egg subsequently laid is not definitely attributable to the effect of the removal of the gland.

Soli reported that 15 or 20 days following this operation the fowl's calcium metabolism becomes deranged, and that this was indicated by the production of eggs in which the shell was either totally or partially suppressed; after another short period (at about 40 days) there was a return to a normal calcium metabolism and the production of eggs with normal shells.

Determination of blood calcium of experimental and control females was made by Professor F. B. Hutt* to whom our thanks are due for the data of Table I. Fourteen control females upon which determinations of blood calcium were made during the same period showed an average calcium content of 18.65 mg. per 100 cc. of serum. In spite of the fact that these measurements were made at a time when (on the basis of Soli's experiments) the greatest effect following deprivation of the thymus was to be expected, the

[1] Soli, U., *Pathologica, Rev. quindic. (Genova)*, 1911 **3**, 118.

* Professor Hutt, now of the University of Minnesota, was at the time of this experiment, in the Animal Breeding Research Department of the University of Edinburgh.

TABLE I.

Number of Bird	Days Postoperative	Amount of calcium in mg. per 100 cc. serum
29	17	25.70
	28	22.45
39	18	29.55
	29	28.40
94	15	24.85
	26	26.60

operated birds in this period showed a somewhat higher blood calcium content than did the normal laying hens.

The thymectomized birds were killed at the conclusion of the experiment and the neck region carefully examined for persistent nodules of thymus tissue. In No. 94 the operation was apparently successful in that no persistent tissue was present. In 2 birds (Nos. 29 and 39) a tiny fragment of thymus was found anterior to the thyroid; additional fragments of tissue from the post-thyroid region also proved, on histological examination, to be thymus. At the time of killing these 2 birds possessed a total of 0.095 and 0.030 gm. of thymus tissue, respectively; an average of 0.592 gm. of thymus was obtained from a series of 12 normal females of the same age group.

The frequent occurrence of thymus tissue near and posterior to the thyroid was further indicated by a careful study of the 12 control birds, all but 2 of which showed definite amounts of typical tissue in this region. The distribution of this tissue is somewhat variable; besides being present within the capsule of either one or both thyroid lobes (and penetrating into the substance of the gland) in 3 cases it was found closely apposed but external to the capsule—extending around the posterior end of the thyroid and coming to lie between it and the parathyroid gland. Terni[2, 3] has reported "thymus-like tissue" within the 2 post-branchial bodies of the fowl. The results obtained from both the experimental and the normal birds indicate that removal of the thymus lobes as far down as the thyroid gland would result in a completely thymectomized bird only in isolated cases.

The data recorded here show that removal of the thymus gland in the hen led neither to the lowering of the calcium content of the blood nor to the production of eggs deficient in shell or egg membranes. Since the operation could be said to be completely success-

[2] Terni, T., *Arch. Ital. di Anat. e di Embriol.*, 1927, **24**.

[3] Terni, T., *Atti Reale 1st Ven. di Sci., let. ed arti*, 1928, **87**, 197.

ful in only one of the birds it might be argued that the evidence presented here is not sufficiently strong to counterbalance Soli's positive results. For the 4 comparable cases quoted by Soli a post-mortem examination was made on only one bird; complete removal was reported. It is perhaps significant that this operated bird again began to produce eggs with normal shells when calcium salts were added to the diet.

We have also thymectomized 9 immature fowls, which subsequently produced eggs with wholly normal shells. This same result has been previously reported for such fowls by Ackert and Morris[4] and more fully by Morgan and Grierson.[5] Riddle and Krizenecky[6] have reported a similar result from thymectomized and bursectomized immature pigeons; they advance the view that even if all of the true thymus is extirpated there still remains in the body much lymphoid tissue possessing thymus function.

It has been shown that in our stock of birds, owing to the occurrence of post-thyroid fragments of thymus tissue in the majority of cases, an operation for the removal of the gland could only be partially successful. Since, as far as we are aware, such a distribution of the thymus has not been previously described it is considered possible that some of the previously recorded cases of complete thymectomy might have possessed residual thymus tissue posterior to the thyroid. If an attempt were made to remove these fragments it is conceivable that, owing to their proximity to the parathyroid gland, a temporary faulty calcium metabolism leading to the production of shelless eggs might be attributable to an interference with the parathyroid.

---

[4] Ackert, J. E., and Morris, M. G., *Anat. Rec.*, 1929, **44**, 209.

[5] Morgan, A. H., and Grierson, M. C., *Anat. Rec.*, 1930, **47**, 101.

[6] Riddle, O., and Krizenecky, J., *Am. J. Physiol.*, 1931, **97**, 343.

5714

The Isolation of Two Isomeric Inactive Cystines.

HUBERT S. LORING AND VINCENT DU VIGNEAUD.

From the Laboratory of Physiological Chemistry, University of Illinois, Urbana.

From a study of inactive cystine, obtained by the catalytic racemization by acetic anhydride of the acetyl derivative according to the method worked out by Bergmann and Zervas[1] for various other amino acids, evidence was obtained of the presence of 2 forms of cystine. With the experience gained in the separation of these 2 forms from this material by the fractionation of their hydrochlorides, the inactive cystine produced by the racemization of l-cystine by refluxing with concentrated acid was next investigated. Recently du Vigneaud and Hollander[2] demonstrated the presence of racemic cystine in this material by actual resolution with isolation of pure dextro cystine. The evidence, however, could not reveal whether or not meso cystine was present. By fractionation of the hydrochlorides we have now been able to actually isolate from this inactive cystine 2 modifications one of which, the less soluble is identical in crystalline form and behavior with racemic cystine formed by a mixture of equal amounts of pure dextro and levo cystine. The crystalline form and particularly the solubility of the hydrochlorides of the 2 isomers differ strikingly. The hydrochloride of the racemic modification is much less soluble and crystallizes in diamond-shaped crystals often with 2 opposite corners cut off, making actually a six-sided crystal yet retaining the distinct diamond-like form. The more soluble modification crystallizes in short blunt prismatic crystals. Furthermore we were able to obtain both free cystines in beautifully crystalline condition. The crystals of racemic cystine appeared to be thick elongated hexagons distinctly different from the typical hexagonal crystals of l- and d-cystine. The more soluble cystine crystallizes in thin parallelogram-like plates and in irregular 6-sided plates. There were also differences in the decomposition points between the hydrochlorides of both modifications as well as between the free cystines themselves.

The phenyl isocyanate derivatives were also prepared, the racemic form melting at 190-191°, the other at 184-185°. A mixture of the two melted at 181°.

[1] Bergmann, Max, and Zervas, L., *Biochem. Zeit.*, 1928, **203**, 280.

[2] du Vigneaud, V., and Hollander, L., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 46.

The formyl derivatives were prepared by a method of Professor Hans Clark,* unpublished as yet. The formyl racemic cystine melted at 190-192° and the formyl derivative of the more soluble form had a melting point of 178-180°. The formyl-l-cystine melts at 187-188°. These derivatives likewise showed different crystalline form and solubility. The formyl racemic cystine was resolvable by means of the brucine salt whereas under the same conditions the other isomeric inactive cystine did not yield to resolution.

The actual isolation of racemic cystine from the inactive cystine completes the proof of the presence of this modification in the material. From the evidence obtained we feel justified in tentatively assigning the meso structure to the more soluble isomer. We are subjecting it, however, to as rigorous a proof as possible. The investigation of these isomers and their derivatives is being continued as well as a study of their availability to the animal body in comparison with dextro and levo cystine.

5715

The Anterior Pituitary Sex Hormone of Normal and Semicastrated Rats.

F. E. EMERY, P. W. BASH AND W. R. LEWIS.

From the Department of Physiology, University of Buffalo.

It is well known that the anterior pituitary gland secretes hormones which are growth stimulators to the reproductive system. This can be easily demonstrated by grafting pituitary glands into sexually immature female rats. The enlarged ovaries produced in this way resemble in weight and histological appearance the hypertrophied ovary of semiovariectomized animals. The evidence presented by Engle and others, recently discussed,[1] shows that the anterior pituitary sex hormone is responsible for the hypertrophy of surviving ovaries and accelerated growth in the remaining testis of young animals.[2] Engle[3] contends that the remaining ovary utilizes the anterior pituitary sex hormone previously used by both ovaries. This would give twice the amount of the hormone to the

* The authors wish to thank Professor Clark for so kindly making available to them his method for preparing formyl-l-cystine.

[1] Emery, F. E., *Anat. Rec.*, 1930, **47**, 300; *Physiol. Zool.*, 1931, **4**, 101.

[2] Lipschutz, A., *J. Physiol.*, 1922, **56**, 451.

[3] Engle, E. T., *Anat. Rec.*, 1928, **37**, 275.

remaining ovary and cause it to about double in weight. Recently one of us (Emery[1]) suggested that the remaining ovary became enlarged due possibly to increased activity or hypersecretion of the pituitary gland following unilateral ovariectomy. Pituitary grafts have been made to determine this point.

The animals used were albino rats and consisted of adult males and females used as donors of the grafts and young immature females used for recipients of the grafts. The donors were of 4 types, normal males and females and semicastrated males and females. The latter groups were operated on at least 2 months before their pituitaries were used as grafts. The recipients received 2 pituitary grafts in the muscles of one hind leg on the twenty-fifth and in the opposite leg on the twenty-sixth day of age. They were killed when 30 days old, and the ovaries and uteri were dissected free from fat and connective tissue and weighed. The Fallopian tubes were weighed with the uterus and the latter was cut from the vagina just anterior to the cervix.

The results show that the average weights of the ovaries and uteri of the recipients were almost identical when grafts from either normal or semicastrated donors were used. As shown in Table I,

TABLE I.

Weights of body in gm., ovaries and uteri in mg. All recipients and controls killed at 30 days of age. The females were nullipara.

Donors	Av. body wt.	Recipients							
		Wt. of Ovaries			Wt. of Uterus			Number of rats	Av. body wt.
		High	Low	Av.	High	Low	Av.		
4 normal ♂	222	102	36	50.9	103	58	79.6	10	51.4
4½ cast. ♂	226	115	30	49.6	112	50	79.0	10	54.8
4 normal ♀	175	21	12	16.2	102	36	67.5	20	50.0
4½ cast. ♀	183	23	13	16.8	88	41	64.2	20	48.8
6 normal ♀	165	42	24	34.5	101	49	77.3	6	46.2
6½ cast. ♀	168	41	24	35.7	97	75	84.2	6	46.3
None, controls		20	10	15.5	58	24	36.9	30	50.6

the male pituitary gland is more potent than the female, which is in agreement with others. The 4 female pituitaries grafted in one recipient produced so slight an increase, only about 1 mg. above the normal controls, in the weight of the ovary that a small difference in potency of the pituitary of the normal and semicastrated donors might not appear. Thus 6 female pituitaries were grafted in each rat and again the ovaries of the recipients were almost identical in weight, 34.5 mg. for the normal and 35.7 mg. for the semicastrated donors. The uterus in our experience is a more sensitive indicator

of pituitary potency than is the weight of the ovaries. Yet when the average weight of the uteri was used as an index of potency of the pituitaries there was no evidence that the pituitaries of semicastrated rats were more potent than those of normal rats.

After the grafts failed to show an increase in the anterior pituitary sex hormone of semicastrated rats the blood was then tested for this hormone. Blood serum from the different types of donors shown in Table I was injected in amounts of about 5 cc. daily into immature female rats. In no instance was a positive test obtained. In some cases a total of 40 cc. of serum was given to each recipient. The hormone must be in the blood in greater amounts in semicastrated rats, or how can the remaining gonad receive more of the hormone needed for the hypertrophy? We have in other cases (not yet reported) obtained the hormone from the blood of certain rats. Thus we know it must be there in small amounts even though we have not detected it from the serum of any of the donors shown in Table I.

Summary. The amount of anterior pituitary sex hormone in the pituitaries of normal male and female rats was not noticeably changed after semicastration. This hormone has not been found in the blood serum of normal or semicastrated nullipara females, or in normal or semicastrated males. The need for a greater amount of the hormone in the blood of semicastrated animals is pointed out.

5716

Experimental Production of Nephrosis-like Lesions by Sodium Hydnocarpate.

CHESTER N. FRAZIER.

From the Division of Dermatology and Syphilology, Department of Medicine, Peiping Union Medical College.

A 0.1% aqueous solution of a preparation consisting of a selected fraction of the sodium salts of the total fatty acids of hydnocarpus oil was injected intravenously into 6 normal albino rabbits over a period of approximately one year. Injections were given at biweekly intervals into the marginal ear veins. The individual dose was calculated upon the basis of 0.0166 gm. of the sodium hydnocarpate preparation per kilo of body weight, and ranged from 0.022 gm. to 0.03 gm. (0.22 cc. to 0.3 cc. of the aqueous solution). Each animal received a total of 101 injections.

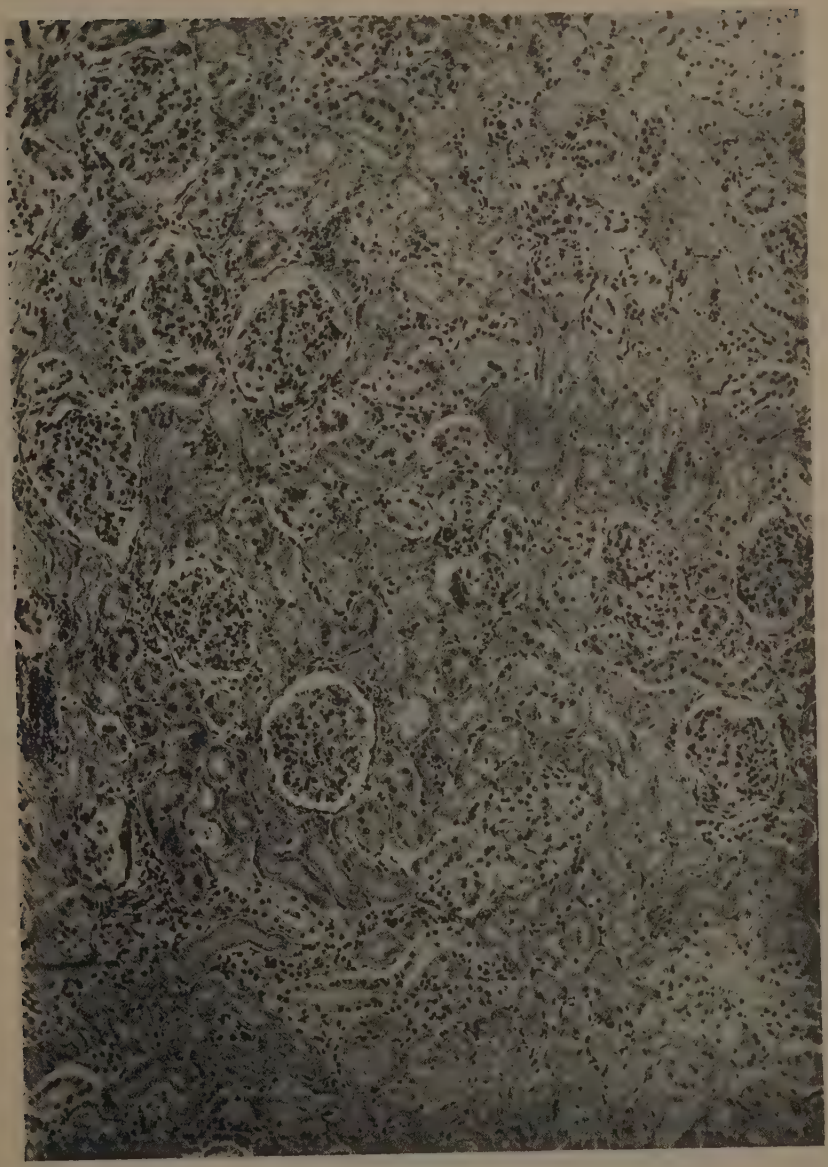

FIG. 1.

Showing non-inflammatory, tubular degeneration in kidney of a rabbit following administration of sodium hydnocarpate. Hematoxylin and eosin. × 156.

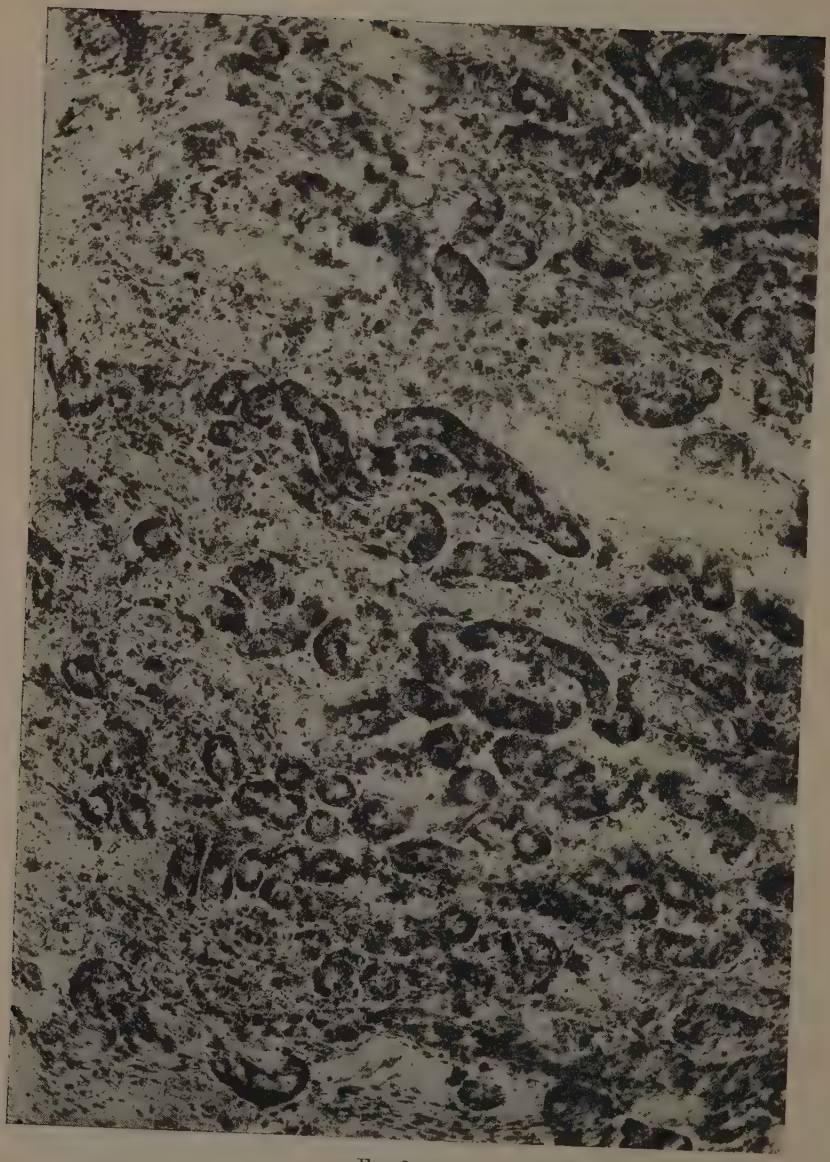

FIG. 2.

Showing fatty degeneration of tubular epithelium in kidney of a rabbit following administration of sodium hydnocarpate. Sudan III. × 156.

During the period of observation no clinically detectable reactions to the drug occurred in any of the rabbits. At the end of this time 2 animals died, one on the day following the last injection and the other 3 days after injection. The remaining 4 animals were killed 5 days following completion of treatment. The chief pathological findings were limited to the liver and kidneys with the exception of the 2 rabbits that died. In one of these there was in addition marked pulmonary edema and in the other diffuse lobular pneumonia with considerable edema of the pulmonary tissues. Free fluid was not present in any of the serous cavities of the 6 animals, nor was there any detectable edema of the skin or subcutaneous tissues.

The liver in each case showed varying degrees of fatty infiltration, especially around the central veins where there was also, in a few instances, evidence of beginning necrosis of the parenchymal cells.

At the time of autopsy no significant gross renal abnormality was noticed. Later study of the fixed specimens in comparison with the fixed kidneys of normal rabbits revealed definite changes in color. In the cortex both on the surface and on section there were irregular pale gray areas. The capsules stripped easily and left a smooth renal surface. The general anatomical markings were unaltered, but it was the impression that the kidneys were slightly smaller than in normal animals.

The microscopic renal changes were striking in all 6 of the animals and are of sufficient interest and importance to warrant this brief description of their nature. The general organization of the cortical and medullary structures remained unaffected. The glomeruli were essentially normal both as to capsule and capillary tufts. The capsular spaces in some instances contained a small amount of homogeneous protein precipitate, but there was no evidence of inflammation. An occasional red blood cell was observed, but these were very few. Adhesions between tuft and capsule were not found. The epithelium of the convoluted tubules in particular showed varying degrees of degeneration (Fig. 1). In many of the tubules there was complete absence of cellular structure and in places the lumen was dilated. The convoluted tubules were filled with casts consisting chiefly of a homogeneous substance and to a less extent of epithelial cells and debris. Where the degenerative process was less advanced desquamation of tubular epithelium was conspicuous. The tubules of Henle and the collecting tubules, although involved in places, were not affected in any comparable degree with the convoluted tubules. In most cases the degenerated changes were not uniformly diffuse, but were limited more or less to irregular areas or

sections of the cortex. No unmistakable sign of inflammation was present in the cortical substance. The arteries and arterioles were normal.

Sections stained with Sudan III revealed heavy fatty infiltration or degeneration of the tubular epithelium (Fig. 2). Lipoids were also seen in the desquamated cells and tubular casts. To a much less degree than in the tubules there was fat staining material in the glomerular tufts. In the case of the animal that died 3 days after the last injection of sodium hydnocarpate numerous minute fat globules were found lying free in the blood vessels. Lipoid degeneration as indicated by the presence of doubly refracting lipoids could not be demonstrated by the polarization method.

In summary it can be said that non-inflammatory, degenerative tubular lesions of the kidney closely similar morphologically to those of nephrosis in man have been produced experimentally through the action of dilute solutions of a soap consisting of the more water soluble sodium salts of the unsaturated fatty acids of hydnocarpus oil. The method is presented as a technique which may be applicable to the study of nephrosis experimentally.

5717

The Effect of Cortin in Asthenia.*

FRANK A. HARTMAN AND GEORGE W. THORN.

From the Department of Physiology, University of Buffalo.

We have studied the effect of cortin in the asthenia of many clinical conditions including Addison's disease. These findings have been compared with observations on normal individuals.

In asthenia there is increased susceptibility to fatigue although the dynamic power of the muscle may not decrease materially. Therefore the measurement of asthenia should test the threshold for fatigue rather than the strength of a single contraction. An ergometer can be successfully used to measure the fatigue threshold provided certain precautions are observed. We employed the ordinary type which uses the middle finger to lift a weight. A load is chosen which will cause definite fatigue in 3 to 5 minutes when lifted every 2 seconds. If too heavy the error due to variation from

* Aided by a grant from the National Research Council.

one test to another may be large. The intervals between tests are great enough to eliminate the practise factor. The base level for fatigue shows less variation in many asthenic individuals than in normals. In all subjects it is necessary to avoid unusual exercise as this may increase the threshold for fatigue by practise on the one hand or decrease it if the exercise is too strenuous on the other.

Four normal individuals have been tested at frequent intervals before and after the injection of cortin. Many others have been tested without the injections. Occasionally in normals, the day to day variation is so great that it is almost impossible to obtain a consistent base level for fatigue. All normals showed an increase of 20 to 50% in the power to do work before fatigue developed, within 1 to 4 days after cortin was begun. The high point came several days after cortin was started, often after the latter had been discontinued. Two showed a maximum increase of 50%; one an increase of 125% and the fourth reached 500%. In some normal individuals a more immediate subjective effect is noted. In an hour or less after the injection the subject feels sleepy and if he yields to the desire to sleep it is unusually sound and restful. Even though he does not sleep, in a couple of hours the feeling of drowsiness passes and he experiences a sense of increased well being and mental alertness which usually lasts for 2 or 3 hours. These effects appear to be more pronounced if one is tired or recovering from a minor infection.

The effect of cortin on the fatigue point has been studied in 6 cases of Addison's disease, 2 in which the cortical insufficiency was almost absolute and the other 4 in which it was less marked. The power to do work without fatigue was increased 700% in one of the severe cases and none in the other thus far. One of the less severe cases has shown no increase, 2 have shown 400% and the third 4900% increases. The maximum often came a few weeks after treatment was started, sometimes many days after it had been stopped. Increase in power to work without fatigue is associated with general improvement, although in some instances raising of the fatigue threshold precedes definite subjective sensation of improvement. When remission follows cessation of treatment the threshold for fatigue falls. The doses employed in these cases ranged from 150 gm. to 3000 gm. cortex daily.

The vomiting, muscular weakness and increase in pigmentation, in some cases of pregnancy indicate adrenal insufficiency. One of the worst cases which Dr. Irving W. Potter has seen was treated with cortin. She was 8 months pregnant, appetite poor, weight

had fallen from 160 to 105 lb. She was so weak that during the day she often had to lie down and had frequent fainting spells. After a few days' treatment, she gained over 10 lbs., appetite was much better, she could be up all day and there were no fainting spells. Blood pressure rose from 78 to 95 mm. systolic.

A case of Graves' disease showed an increase of 200% during the treatment (300-400 gm. of cortex per day) and a marked increase in the sense of well being. These improvements have persisted (3 months since the extract was discontinued).

A case of muscular dystrophy reached a maximum increase of 2700% 3 weeks after the extract was discontinued (the product of 300 to 400 gm. of cortex was injected daily for 14 days). No subjective change was noted for several days except that the patient could sleep better and felt more rested afterward. After 4 months without treatment the fatigue threshold returned toward the old level. With this patient and with others, including normals that show an increase in the power to work, there is no consciousness of a greater ability to work until the test is under way.

Two cases of osteomyelitis in children have been treated. One showed a 300% increase in working power and the other 700%. One month before treatment the latter patient had had scarlet fever.

Marked improvement has been produced by cortin (300 gm. cortex daily for 8 days) in persistent asthenia following diphtheria ("bull neck"). For weeks the patient lay in his bed. In 2 days after cortin was instituted he was sitting up and much brighter. Nine days after the extract was discontinued his power to do work before fatigue had been increased 130%. He steadily improved and finally went home.

A case of rather marked asthenia of possibly neurasthenic origin showed no definite improvement from cortin (as much as 100 gm. of cortex per day).

We have treated a case of *Myasthenia gravis* with large amounts of cortin (600 to 900 gm. of cortex) without effect. Drs. Bassett and Garvey of the University of Rochester have used smaller doses in a similar case without result.

The positive effects obtained in such widely varying clinical cases as well as to a minor degree in some supposedly normal individuals indicate a pharmacological action of cortin.

5718

Staining Treponema Pallidum With Alcoholic Silver Nitrate.

R. T. BOTHE AND H. A. DAVENPORT.

From the Department of Anatomy, Northwestern University Medical School.

An alcoholic solution of silver nitrate has been found to be an efficient stain for nerve fibers in celloidin sections (Davenport[1]). The results obtained on nerves suggested the possibility of staining *Treponema pallidum*, since the methods of Jahnel and of Levaditi[2] employed aqueous silver solutions.

Lymph taken from primary and secondary syphilitic lesions was the material chosen for experimentation. With the exception of one instance in which the lesion occurred on the lower lip, all material was obtained from lesions on the external genitalia.

The lesion was cleaned with gauze and cotton. The exuding lymph was placed on a slide by means of a sterile wire loop and allowed to dry in air at room temperature. The staining procedure was essentially that previously reported (Davenport[3]). The slides were coated with a 2% solution of celloidin, dried for about 2 minutes, and put into a 10% solution of silver nitrate in 85% alcohol for 5½ hours at 37°C. The preparations were reduced in an alcoholic solution containing 3% pyrogallic acid and 5% formalin, and were subsequently gold toned.

Of 10 cases examined, preparations from 4 showed many deeply stained spiral forms having the morphology characteristic of *Treponema pallidum*. These also showed *Treponema pallidum* upon dark field examination and blood taken from the 4 patients gave positive Wasserman and Kahn reactions. No such characteristic forms could be found in smears from the 6 remaining cases, nor could *Treponema pallidum* be observed in dark field examinations.

Our results lead us to believe that alcoholic silver nitrate is an efficient, rapid and simple method of staining *Treponema pallidum* in smears.

---

1 Davenport, H. A., *Anat. Rec.*, 1929, **44**, 79.

2 Cited from Spielmeyer, W., *Technik der Untersuchung des Nervensystems*, Berlin, 1930, 154.

3 Davenport, H. A., *Arch. Neurol. and Psychiatry*, 1930, **24**, 690.

5719

Early Behavior of Embryos of the Turtle, Terrapene carolina (L).

HIDEOMI TUGE. (Introduced by G. E. Coghill.)

From the Wistar Institute of Anatomy and Biology, Philadelphia.

Male and female specimens of *Terrapene carolina* (L) were collected early in the breeding season and kept in pens. The times of egg-laying were observed, and the eggs were removed from the nest within a few hours after they were laid. Each clutch of eggs was planted separately and records kept of the time of laying. From these eggs 40 living embryos were procured, upon the basis of which this report is made.

It was found that the age of the embryo as calculated from the time of egg-laying could not be depended on as an index of the degree of development. Embryos of the same clutch in some cases differed considerably in this respect; and great difference appeared between clutches of the same age. Obviously eggs develop in the oviduct of this species for various periods. It was largely a matter of chance, therefore, in selecting eggs for study with reference to particular phases of development; although an embryoscope improvised upon a 6-volt, 108-watt, ribbon filament Mazda lamp was of considerable assistance in approximating the age desired.

Spontaneous movements occur, apparently for at least several hours, before movement can be elicited in response to touch. Embryos with carapace-length of 6 mm. respond to touch on the snout, but not to touch on other parts, except possibly on the shoulder on very rare occasions (probably spontaneous movement in these instances). The movements at this time always involve the head and neck, and usually, also, the trunk, tail, and fore and hind limbs. The limbs never move in this phase of development without movement of the trunk. These reactions are total reactions, inasmuch as a more caudal or distal part does not move without movement of the more anterior or proximal part. Since the limbs are at this time insensitive to touch there are no limb reflexes. In embryos with carapace-length of 7 to 7.5 mm., movements of the limbs, (fore and hind) occur in response to touch on the limbs, without perceptible movement of the trunk. These movements are local reflexes. While there were suggestions of total reactions only in response to touch on the appendages in some embryos, this pattern has not yet been definitely established as representing a definite

phase in the development of the behavior pattern. But it is demonstrated that in the turtle the limbs move as a part of a total behavior pattern before they move discretely in response to local stimulation. This is of particular interest because in the adult of this species the trunk, being anchored to the carapace, is not an active factor in locomotion, which is effected wholly by the appendages.

The specimens were photographed and preserved for correlated anatomical study.

5720

Reticulocytes.

EDWIN E. OSGOOD AND MABLE M. WILHELM.

From the Departments of Medicine and Biochemistry, University of Oregon Medical School, Portland, Oregon.

In an effort to adapt the reticulocyte stain for use with oxalated venous blood and incorporate it in our uniform system of hematologic methods,[1] 7 methods of reticulocyte staining in current use were tried. None proved wholly satisfactory. Experiment showed that increasing the time of exposure or increasing the concentration of the dye increased the number of reticulocytes found. It was also found that the presence of 2 mg. of potassium oxalate per cc. of blood did not alter the reticulocyte count.

The following procedure was found to give uniformly good stains with the maximum number of reticulocytes in a given blood:

Mix equal parts (5 drops) of oxalated venous blood[1] (or fresh blood) and 1% brilliant cresyl blue in 0.85% NaCl solution in a small test tube and allow to stand one minute or more. Mix and make a thin smear. This may be counted when dry, or counterstained with Wright's stain. Count all the red cells (preferably with a hand tally) in an oil immersion field and then count all the reticulocytes in that field. Move to an adjacent field and repeat until 1000 red cells have been counted. If the count is more than 5%, only 500 cells need be counted. The counterstain is necessary if the slide is to be kept for more than 48 hours.

This method has many advantages. It is very simple and convenient and consistently gives a higher reticulocyte count than the

[1] Osgood, E. E., Haskins, H. D., and Trotman, F. E., *J. Lab. and Clin. Med.*, 1931, **16**, 476.

other methods tried. The stain keeps indefinitely and need not be filtered before using. The oxalated blood may stand for as long as 48 hours before the count is made. Overstaining does not occur even though the smears are not made until 2 hours after the stain and blood are mixed. The reticulocytes are clearly and deeply stained and the red cells are neither crenated nor distorted. The smears keep indefinitely if counterstained with Wright's stain.

A reticulocyte stain on oxalated blood which had stood for 144 hours and was badly hemolyzed showed the same percentage of reticulocytes as did the fresh blood. This suggested to us that perhaps this is not a vital stain as is generally stated, but is merely a dye which will not stain fixed cells. To investigate this point further, equal parts of blood and 1% sodium cyanide solution were mixed and allowed to stand from 20 minutes to 1 hour and reticulocyte counts were done. These gave practically the same results as counts on the original blood. Cells fixed by various methods failed to show any reticulocytes.

Normal values for reticulocytes will have to be established by this method. Preliminary studies suggest that bloods of healthy adults will show about 2% reticulocytes.

5721

The Phosphatase Content of Fractured Bone.*

R. M. MCKEOWN† AND J. I. OSTERGREN. (Introduced by S. C. Harvey.)

From the Department of Surgery, Yale University School of Medicine.

In a recent paper Kay[1] states that he observed an increase in plasma phosphatase following fracture, and indirectly he suggests a similar rise in bone phosphatase. In view of our findings on the breaking strength of fractured fibulae of rats,[2] we were of the opinion that a positive correlation existed between the breaking strength and bone phosphatase. This we sought to demonstrate. It was also believed that if the activity of phosphatase in the healing process of fractures is antagonized, after the primary callus has formed on the fifteenth

*The expenses of this investigation were defrayed by Davis and Geck, Inc.

† Davis and Geck Fellow in Surgery.

[1] Kay, H. D., *J. Biol. Chem.*, 1930, **89**, 249.

[2] McKeown, R. M., Lindsay, M. K., Harvey, S. C., and Lumsden, R. W., *Arch. Surg.*, in press.

day, by the hormone of the parathyroid gland, as we suggested elsewhere,[2] that subsequent to the fifteenth day the bone phosphatase should be reduced.

The method of selecting, feeding and fracturing the right fibulae of the albino rats has been described.[2] The bone phosphatase of the combined unfractured left fibula and tibia, as well as the bone phosphatase of the combined fractured right fibula and its tibia, were estimated by a method personally suggested by Kay,[1] the details of which will be published later. The results are tabulated in Table I.

TABLE I.
Average of Phosphatase Units Obtained.

Post-Operative Day	No. Animals	Right Fractured	Left Unfractured
2	2	17.7	15.6
4	2	13.3	13.9
6	3	19.1	18.0
9	3	22.8	24.0
12	2	16.5	15.1
15	4	19.2	19.8
18	2	13.9	13.5
21	2	9.3	9.6
24	3	16.8	15.3
27	2	8.2	9.3
30	2	13.1	11.0
33	2	7.0	6.9
36	2	7.2	5.9
39	2	16.8	14.6
42	2	8.5	9.1
45	2	11.0	10.2
	Controls (entirely free of fractures)		
0	6	12.8	13.6

Our findings, incomplete as they are, strongly indicate that the level of bone phosphatase in the fractured fibula rises with the formation of the primary callus and falls as the medullary space is developed. The rapid loss of callus strength during the formation of the medullary space, between the fifteenth and twenty-first days, has been suggested elsewhere as being due to a powerful circulatory decalcifying substance which may possibly be the parathyroid hormone.[2] The sharp reduction in the phosphatase content of the fractured right fibula and its normal tibia, subsequent to the formation of the primary callus on the fifteenth day, and during the formation of the medullary cavity, suggests that an actual antagonism does exist between phosphatase, and this at present unknown decalcifying substance.

The relatively close agreement between the phosphatase content of the unfractured left fibula and its normal tibia in the left leg, to

that observed in the fractured right fibula and its normal tibia in the right leg, offers additional proof for our previous reasoning, from the results of breaking strength determinations,[2] that the repair of a fracture is not as local as was formerly thought, and that the skeleton at large is called upon to furnish in a remarkably short space of time those substances essential for the healing of the fracture.

5722

Viability of the Cells of Rous Chicken Sarcoma Desiccate.

FRANK A. MC JUNKIN.

From the Department of Pathology, Loyola University School of Medicine.

It is a common experience in preparing tissue cultures, in transplantation and in supravital staining that desiccation is rapidly fatal to mammalian cells. It has usually been assumed that the tissues of all higher animals are similarly injured. Nakahara,[1] however, found in the desiccate of the Rous chicken sarcoma cells that he thought to be alive. He treated the cells of a desiccate more than 4 months old with dilute solutions of trypan blue and also inoculated fresh chicken plasma with them. By both methods cells thought to be living were seen.

Since Nakahara apparently observed the cultures for only a short time (3 or 4 hours) it seemed desirable to keep cultures under observation for a longer period. A preparation of desiccate was generously furnished me by Dr. Rous. It was prepared November 16, 1925, and on June 2, 1926, several dozen cultures were made employing the usual cover-glass method. The desiccate was first rubbed up with a small amount of Ringer solution to make a thick paste which was kept on ice until the explants were made. In one series whole fresh Barred Rock plasma was inoculated with the paste and in another the plasma was diluted with one-third volume of distilled water. The cultures were at once placed in the incubator and examined at 3 and 5 hours, one, two and five days. Round cells of medium size were seen on first examination and suggested growth. The nuclei were perfectly distinct and their irregular outline might be interpreted as evidence of ameboid motion. Many of these cells were located by ink marks on the cover-glass. After 5

[1] Nakahara, W., *Gann. Japanese J. Cancer Res.*, 1926, **20**, 13.

days there was no change in position or outline of these cells. In places at the periphery of the explant spindle cells projected outward radially into the plasma. These likewise remained stationary during the 5 days of incubation. On June 2, three hens were inoculated with the same paste from which the cultures were made. The hens were mongrels having atypical Barred Rock markings. Two of them developed large tumors the latter part of July. July 30 a desiccate was made from one of these by drying over phosphorus pentoxide in partial vacuum at a temperature below 32°C. On September 21, 1926, when completely dry this was sealed in small tubes, In early June, 1927, several baby chicks were inoculated in the pectoral muscle and those that lived one month developed tumors. At this time explants to heparinized chicken plasma were made and clotting produced with embryo extract. The results were the same as those obtained with the original desiccate.

The fresh living cells of the Rous sarcoma both round and spindle-shaped when examined directly with dilute neutral red react with the appearance of characteristic cytoplasmic granules.[2] Many examinations of the desiccate were made by this method. Cytoplasmic granulation appeared in none of the cells. Although there were differences in the depth of staining both nuclei and cytoplasm tended to stain faintly and diffusely.

Conclusions. In 2 desiccated preparations of Rous chicken sarcoma viable cells were not demonstrable by the tissue culture method. By the method of supravital staining with neutral red the reaction of the desiccate was unlike that given by the living sarcoma cells.

This work was begun in the Department of Pathology, Washington University, St. Louis, Missouri, and I am indebted to Professor Leo Loeb for the privilege of publishing those results along with the ones obtained here.

[2] McJunkin, F. A., *J. Cancer Res.*, 1926, **12**, 47.

5723

Electrical Potential Difference Across the Nuclear Membrane of the Starfish Egg.*

SAMUEL GELFAN.

From the Department of Physiology and Pharmacology, University of Alberta, and the Marine Biological Laboratory, Woods Hole.†

An electrical potential difference has been demonstrated to exist across the membrane of the intact germinal vesicle of the starfish egg *(Asterias forbesii)*. For the measurement of this P. D. non-polarizable microelectrodes (Ag-AgCl-sea water) 1-3μ in diameter, a potentiometer and a highly sensitive moving coil galvanometer were used. The eggs were placed in a hanging drop of sea water over a moist chamber, and the electrodes were introduced into the egg by means of a micromanipulator. The measurements were accurate to within one millivolt.

In attempting to enter the germinal vesicle, an invagination at the point where the pressure was applied by the microelectrode, and general flattening of the nucleus would be produced. As the latter was pierced by the electrode, the invagination and flattening, however, would suddenly disappear, the nucleus immediately resuming the original spherical form. This physical reaction constituted the visual criterion for the penetration of the germinal vesicle. Corollated with this criterion was the instantaneous deflection of the galvanometer as the electrode penetrated the nucleus.

The interior of the nucleus was always positive with respect to the cytoplasm or the sea water medium. The magnitude of the potential across the nuclear membrane varied from 4 to 21 mv., the average of over 100 recorded measurements being 10 mv. The size of the potential depended considerably upon the condition of the animals and the eggs. In eggs that were in relatively poor condition the potentials varied from 5 to 10 mv., whereas the measurements of eggs in good condition varied from 15 to 20 mv. The potentials were fairly constant in any series of measurements. The variations were rather in different animals collected at different times. The degree of maturation of the eggs, and the injury produced by the

* These experiments were performed two years ago when the author was a Donnelly Research Fellow in Physiology at the University of Chicago.

† The author is grateful to Dr. G. H. A. Clowes for his kindness in granting the use of the Eli Lilly Co. laboratories at the Marine Biological Laboratory for these experiments.

insertion of the microelectrodes into the nucleus also influenced the magnitude of the P. D. In a normally maturating egg the P. D. disappears as the membrane breaks down. These experiments clearly indicate that the intactness of the germinal vesicle is essential for maximal voltage.

If maturation of the eggs were inhibited, according to the method described by Lillie,[1] by placing them for 70 seconds in sea water at a temperature of 32°C (controls at this time maturating 95-100%), uniform potentials varying from 15-20 mv. were obtained.

With one electrode inside the germinal vesicle, the magnitude of the potential was the same, whether the other electrode was in the cytoplasm or in the sea water. It is also interesting to note that in many experiments in which one electrode was in the cytoplasm and the other in sea water, no measurable potential could be demonstrated between the cytoplasm of this egg and the sea water during all stages from immaturity to the second cleavage stage.

Varying the hydrogen ion concentration of the sea water from pH 8.2 (normal) to pH 6.0 had no significant effect on the electrical potential across the germinal vesicle membrane as compared with the controls. However, when the KCl concentration of the sea water (maintaining isotonic conditions) was increased, the magnitude of the potential was reduced. This depression was most pronounced in a mixture of ½ sea water and ½ isotonic KCl (pH 8.0); in this medium the P. D. was only 10-20% of the control measurements.

5724

Purification of Poliomyelitis Virus by Adsorption and Elution.*

ALBERT B. SABIN. (Introduced by W. H. Park.)

From the Department of Bacteriology, New York University, and Bellevue Hospital Medical College.

The exact nature of filterable viruses is considerably obscured by their close association with substances derived from the tissues in which the virus is found. The purpose of this communication is to describe a method whereby the virus of poliomyelitis

[1] *J. Exp. Zool.*, 1908, **5**, 375.

* Supported by a grant from the International Committee for the Study of Infantile Paralysis.

may be partially purified, and ultimately perhaps be obtained in its pure state. The method follows closely the procedures which enabled Willstätter and his coworkers to isolate enzymes in their purest known form. Numerous investigators[1] have reported the capacity of various suspensions to adsorb filterable viruses. The adsorbed viruses are in most instances inactivated, and in the case of poliomyelitis, Amoss[2] states that "the presence of colloidal substances with adsorptive power destroys the virulence after 1 or 2 days." Rhoads[3] described the adsorption and inactivation of poliomyelitis virus by aluminum hydroxide, type "C" of the Willstätter series, and showed that adsorption occurred at pH 5.5 and 7.0, but not at pH 8.8. Gildemeister and Herzberg[4] succeeded in adsorbing bacteriophage on kieselguhr and subsequently eluting it with dilute ammonia. Kligler and Olitzki[5] confirmed these observations on bacteriophage, and were able to do the same with fowl-pox virus, using kaolin as the adsorbing agent.

If the inactivation of the poliomyelitis virus is not irreversible, it should be possible to adsorb it at an acid pH, and elute at an alkaline pH. For adsorption of the virus I used alumina gel "C", prepared according to the method of Willstätter and Kraut[6] excepting that the centrifuge was used, instead of natural sedimentation. An effective gel was thus obtained, the process requiring only 2 days as compared with 2 weeks or more in the original procedure. The gel was standardized to contain 22-25 mg. Al_2O_3 per cc. The poliomyelitis virus† was a Seitz-filtrate of a 5% monkey cord suspension. The procedure in a typical adsorption-elution experiment was as follows: To 5 cc. of alumina gel "C", 1 cc. of M/15 KH_2PO_4 and 5 cc. of 5% virus filtrate were added. Immediate flocculation of the gel occurred. The mixture was shaken for 20-30 min. and left in the refrigerator for 3-4 hours. It was then shaken again and centrifuged for 20 min., or until the densest possible packing of the gel occurred. The supernatant liquid which had a white (lipoid-containing) "cake" at its surface was poured off. The sides of the tube and the surface of the gel were carefully washed with

[1] Levaditi, C., and Nicolau, S., *Compt. rend. Soc. biol.*, 1923, **88**, 66. Lewis, M. R., and Andervont, H. B., *Am. J. Hyg.*, 1927, **7**, 505.

[2] Amoss, H. L., in "Filterable Viruses," ed. by Thos. Rivers.

[3] Rhoads, C. P., *J. Exp. Med.*, 1931, **53**, 399.

[4] Gildemeister, E., and Herzberg, K., *Centbl. f. Bakt.*, Orig. I, 1924, **91**, 228.

[5] Kligler, I. J., and Olitzki, L., *Brit. J. Exp. Path.*, 1931, **12**, 172.

[6] Willstätter, R., and Kraut, H., *Ber. Chem. Ges.*, 1923, **56**, 149.

† I am very much indebted to Drs. Brebbner and Weyer for supplying me with poliomyelitic monkey cords, and their interest in this work.

distilled water. The sediment was now mixed with 5 cc. of M/15 Na_2HPO_4. The gel which was formerly flocculated became homogeneous; after shaking for 20 min., it was left at room temperature or in the refrigerator over night. The following morning it was again shaken and then centrifuged. The supernatant liquid was colorless and water-clear without any "cake" at its surface. The original virus filtrate, the adsorbed supernatant liquid and the eluate were then diluted to the same volume. One cc. of the dilutions (equivalent to 0.05 cc. of the original) was injected intracerebrally into monkeys. The results are shown in Table I.

TABLE I.
Adsorption and Elution of Poliomyelitis Virus.

Monkey No.	Solution Tested	Dose cc.	Result
1	5% Virus filtrate—"593"	0.05	Typical poliomyelitis, 6 days
2	Adsorbed supernatant liquid	0.05	No symptoms
3	M/15 Na_2HPO_4 Eluate	0.05	Typical poliomyelitis, 5 days
4	5% Virus filtrate—"590"	0.05	Typical poliomyelitis, 6 days
5	Adsorbed supernatant liquid	0.05	No symptoms
6	M/150 Na_2HPO_4 Eluate	0.05	Typical poliomyelitis, 10 days

It may be seen that with the doses used, the adsorption of the virus is probably complete, and that the M/15 Na_2HPO_4 eluate, volumetrically equivalent to the original virus filtrate, produced poliomyelitis even more rapidly than the control. When M/150 Na_2HPO_4 was used for elution, the injected monkey developed poliomyelitis 4 days later than the control. Although one cannot yet definitely state that differences in the incubation period are indicative of the virulence or quantity of virus, I have the feeling that the M/15 Na_2HPO_4 is the more effective eluting agent.

Preliminary quantitative determinations indicated that 80-90% of the organic constituents of the original virus filtrate remained in the inactive adsorbed supernatant liquid, and that not more than 10% was released from the gel during elution. The eluted virus solution had no coagulable substances and failed to give a Biuret or Ninhydrin reaction, but since Seitz-filtrates of 5% monkey cord and brain suspensions, which yield an appreciable amount of heat-coagulable material, are either Biuret-negative or only faintly positive, these chemical tests are of little value here. The eluted virus solution also failed to give a precipitin reaction with the serum of 2 horses which had been under immunization for a long period of time with poliomyelitic monkey cord and brain; but this again does not exclude the presence of neuro-proteins since the

precipitin content of these sera for concentrated suspensions of normal or poliomyelitic monkey cord is extremely low. Similarly there was no complement fixation in a mixture of eluted virus and antipoliomyelitic horse serum. However, I consider the eluate as well as the original filtrate to be very dilute solutions (suspensions) of virus, and am postponing a more detailed chemical and immunological study of the virus to the time when I shall have purified and concentrated a large quantity of it.

5725

Vaccination of Humans against Yellow Fever with Immune Serum and Virus Fixed for Mice.

W. A. SAWYER, S. F. KITCHEN AND WRAY LLOYD.
(Introduced by T. P. Hughes.)

From the Yellow Fever Laboratory of the International Health Division, Rockefeller Foundation, New York.

The method of vaccination which we have found effective in immunizing human beings against yellow fever is based on the well known fact, reported by other investigators and frequently observed in our laboratory, that monkeys inoculated with highly virulent strains of yellow fever virus and given simultaneous or preceding protective injections of yellow fever immune serum are found to have a solid active immunity after the passive immunity has disappeared. As accidental infections of laboratory workers with the virulent strains of virus maintained in monkeys have caused many serious illnesses and 5 deaths,[1] we have substituted for such virus in the vaccine a less dangerous strain which was established in mice by Theiler,[2] through intracerebral inoculations, and has been passed successively through more than 100 of these animals. Although this virus has lost the power to kill monkeys on subcutaneous inoculation, animals so inoculated frequently show fever and we consider necessary the simultaneous injection of immune serum with the vaccine. Moreover, the 3 recorded[1] accidental infections of man with yellow fever virus fixed for mice resulted in definite though mild attacks of yellow fever.

The vaccine is prepared in 2 parts. (a) A 10% suspension of

[1] Berry, G. P., and Kitchen, S. F., *Am. J. Trop. Med.*, in press.
[2] Theiler, M., *Ann. Trop. Med. and Parasit.*, 1930, **24**, 249.

mouse-brain tissue containing yellow fever virus (French strain, more than 100 passages through mice) in fresh, sterile, human immune serum was prepared, tested for sterility by making cultures, and centrifuged. The supernatant fluid was passed through Seitz or Berkefeld N. filters, tubed in 1 cc. portions, dried in the frozen state by a method previously published,[3] stored in sealed tubes in the refrigerator, and later tested in animals. This part of the vaccine, redissolved in distilled water, had no effect on mice when injected intraperitoneally but caused fatal yellow fever encephalitis when injected intracerebrally. Monkeys vaccinated with this material together with supplementary immune serum remained well and developed immunity. (b) Immune serum for supplementary injections was secured from persons who had recently had yellow fever. To the pooled serum was added 0.2% tricresol. This serum was considered suitable for use if 0.3 cc. per kilogram of body weight given subcutaneously protected monkeys against a simultaneous subcutaneous injection of virulent virus from monkey source.

The dried vaccine was dissolved and brought to the original volume with distilled water. The amount of this fluid injected was 0.03 cc. per kilogram of body weight, and the amount of immune serum 0.3 cc. per kilogram. A small part of the total amount of this serum was in the dried vaccine and the larger part was given in supplementary injections. The supplementary serum was injected subcutaneously in 2 different places in the abdominal wall, and immediately afterwards the redissolved vaccine was injected in the same way.

Ten persons were vaccinated between May 13 and June 29, 1931. The first 4 received a vaccine which was prepared and administered by methods differing somewhat in detail from the description given above. Unlike the later preparations, it had not been centrifuged or filtered and consequently contained much more mouse-brain tissue. As a result there was much soreness of the abdominal muscles at the sites of injection of the vaccine and some local redness and swelling. These manifestations disappeared almost completely during the day following vaccination. The persons later vaccinated received filtered vaccine and had, as a rule, less local reaction. No other symptoms of importance were observed except slight elevations of temperature in some of the cases. At-

[3] Sawyer, W. A., Lloyd, W. D. M., and Kitchen, S. F., *J. Exp. Med.*, 1929, **50**, 1.

tempts to recover the virus from the circulating blood of 3 persons during the first 24 hours after vaccination were unsuccessful.

The production of immunity. Sera were obtained from the vaccinated persons before vaccination and at approximately weekly intervals thereafter, and were tested by the intraperitoneal protection test in mice (Sawyer and Lloyd[4]). All sera were without protective power before vaccination, but in every case protective power against yellow fever virus was demonstrated later. In one case protection appeared for the first time 7 days after vaccination; in two cases 9 days after; in 6 cases 12 to 14 days after; and in one case 21 days after. Sera from 7 persons were tested also in monkeys and gave protection.

5726

Cultivation of the Virus of the Common Cold in Tissue Medium.

A. R. DOCHEZ, KATHERINE C. MILLS AND YALE KNEELAND, JR.

From the Department of Medicine, College of Physicians and Surgeons, and the Presbyterian Hospital, New York.

In a previous report[1] we presented evidence of the cultivation of the virus of the common cold in tissue medium *in vitro*. A single culture was maintained for 15 generations representing duration of life outside the human body of 74 days.

We now report a second cultivation of the virus of the common cold by a technic similar to the one previously described. Naso pharyngeal washings were obtained from a patient within the first 24 hours of a typical acute cold. These were passed through a Seitz filter, the activity of the filtrate ascertained by the intranasal inoculation of apes, and it was planted without concentration in the medium previously described. In this series bouillon made from the special peptone of Dubos was used as diluent instead of Tyrode's solution. The culture was carried under vaseline seal for 17 generations. The total duration of life outside the human body was 73 days. The final dilution of the original material was 1-100 quadrillion.

The average time of transfer varied from 3 days to 6 days. The

[4] Sawyer, W. A., and Lloyd, W., *J. Exp. Med.*, 1931, **54**, 533.

[1] Dochez, A. R., Mills, Katherine C., and Kneeland, Yale, Jr., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 513.

fourth and fifth generations were tested for activity on apes. No infection resulted. The 17th generation representing a dilution of the original material of 1-100 quadrillion was tested for activity on 3 human volunteers. The volunteers were rigidly quarantined under the supervision of an experienced nurse according to the technic previously described. Two volunteers, 3 days after isolation, and one 7 days after isolation received intranasally uninoculated culture medium prepared in the same manner as the medium used for cultivation of the virus and incubated for a similar period of time. Aside from very slight temporary irritation no symptoms of infection resulted from the control inoculations. The appearance of the throats remained unchanged.

Nine days after isolation volunteer No. 1 received into each nares 1.5 cc. of virus tissue culture centrifugalized slowly to remove tissue clumps. Immediately afterwards he was turned on his face for one minute to prevent too rapid swallowing of the material. No symptoms of infection resulted. However, by the second day after inoculation the appearance of the throat had changed, the mucous membrane appearing slightly swollen, somewhat redder than normal and with some swelling of the lymphoid follicles. We have frequently observed this change in the throats of inoculated individuals when definite symptoms of infection have been absent. In spite of these slight changes the result has been considered negative.

Volunteer No. II received 11 days after isolation 1.5 cc. of tissue culture medium into each nares according to the technic employed above. Two days after inoculation the patient awoke with a sore congested feeling in the frontal region, no coughing or sneezing. A few hours later, he vomited, felt listless and suffered from loss of appetite. There was increased redness of the throat, swelling of the lymphoid follicles and a small white patch on the left tonsil. There was a voluminous thin mucous discharge from the posterior naso pharynx. There was no fever. On the next day symptoms of respiratory irritation were absent. However, the objective changes in the throat persisted up to the time of discharge from quarantine 6 days after inoculation. This volunteer experienced a mild experimental cold.

Volunteer No. III was inoculated, in the manner described, 5 days after isolation. One day after inoculation there was slight sneezing. The next day he awoke with nasal obstruction and a mucoid discharge from the nose. Thick mucus was cleared from the throat. He complained of a "splitting headache", the throat showed definite increased redness and swelling of the lymphoid follicles. He grew

worse during the day and vomited his lunch. The eyes became suffused. The third day after inoculation he felt worse and seemed to have infection of the right maxillary antrum. There was post nasal discharge, the throat was redder and the headache persisted though of lessened intensity. The fifth day he was better though somewhat listless. He coughed during the night and raised a good deal of phlegm during the day. There was still nasal obstruction, a slight headache and a mucoid discharge from the nose. He was discharged on the seventh day after inoculation, feeling better. The headache had disappeared. There was still nasal obstruction shifting from side to side, cough and expectoration. The pharynx was still red and swollen. This volunteer suffered from an experimental cold of moderate severity.

Of the 3 volunteers inoculated with the 17th generation of a tissue medium culture of the virus of the common cold 2 exhibited positive results, one experiencing a cold with mild manifestations and the other a cold with moderately severe symptoms. The third with the exception of slight changes in the throat gave a negative result. These experiments confirm the evidence of the cultivation of the virus of the common cold in tissue medium previously reported.

5727

The Depression of the Vomiting Mechanism by Digitalis.

HARRY GOLD, NATHAN KWIT AND JANET TRAVELL.

From the Department of Pharmacology, Cornell University Medical College.

The vomiting produced by toxic doses of the digitalis bodies has been studied chiefly from the point of view of its mechanism and the conditions under which such vomiting occurs. Some time ago the fact was noted that in some instances toxic doses of digitalis which at first caused vomiting, when repeated, failed to cause emesis. The present experiments were planned to extend this observation and to determine under what circumstances the digitalis bodies might produce a depression of the vomiting mechanism.

Observations were made on cats and dogs following the repeated intravenous injection of various members of the digitalis group. Each experiment lasted several days, and in the series of dogs some of the changes produced in the heart by the drug were recorded by frequent electrocardiograms.

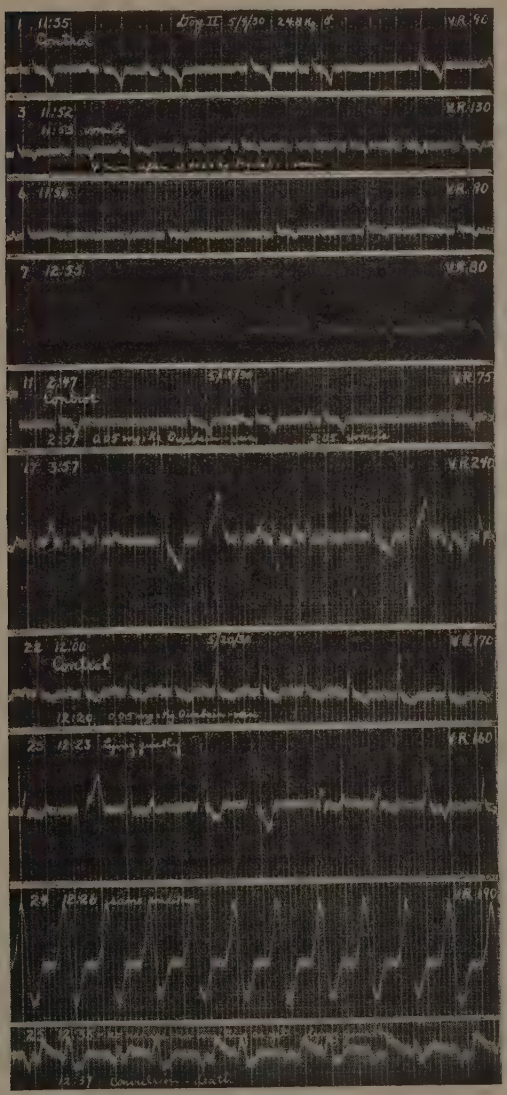

FIG. 1.

Showing relation between vomiting and electrocardiographic changes. The first dose caused vomiting but no ectopic rhythm (tracings 3, 6) and after about an hour the electrocardiogram (tracing 7) was practically identical with the control. Ten days later a dose induced vomiting in addition to ventricular ectopic rhythm (tracing 17). The following day the same dose produced more intense poisoning and death (tracings 23, 24, 25) without vomiting.

It was found that there was a progressive elevation of the threshold of the vomiting mechanism to digitalis. Thus the initial dose of the drug which induced vomiting, when repeated often failed to produce this result, and increasingly larger doses were required to cause vomiting. It was found that the cumulation of digitalis after repeated injections might produce very severe poisoning of the heart as indicated by the development of such toxic rhythms as ventricular tachycardia, while at the same time vomiting did not occur. In fact, in 3 dogs the final increment of digitalis which was fatal failed to cause vomiting, though sufficient time elapsed before death for vomiting to have occurred.

The above facts are illustrated by Fig. 1, which shows selected electrocardiograms from one of the shorter experiments. It may be seen that digitalis given by vein produced vomiting in 4 minutes (tracing 3), and that subsequently an intravenous injection of oubain was followed by vomiting in 8 minutes (tracing 11). On the following day, however, the same intravenous dose of oubain induced a toxic rhythm resulting in death, but failed to cause emesis although 17 minutes had elapsed before the animal died (tracings 23, 24, 25).

Because of the significance attached to the symptom of vomiting as an index of toxicity in digitalis therapy, the possibility of depression of the vomiting mechanism to digitalis apparent in these experiments must be taken into consideration in the clinical use of the drug.

5728

Liver Changes After Deprivation of External Pancreatic Secretion.

B. N. BERG AND T. F. ZUCKER.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

In a recent investigation concerning the physiology of pancreatic secretion[1] a series of dogs was deprived completely of pancreatic juice by means of fistulas made according to the Elman and McCaughan modification of the Rous-McMaster technique[2] and by

[1] Berg, B. N., and Zucker, T. F., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 724. Zucker, T. F., Newburger, M. G., and Berg, B. N., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 666.

[2] Elman, R., and McCaughan, J. M., *J. Exp. Med.*, 1927, **45**, 561.

ligation of the pancreatic ducts. At autopsy, histological examination of the livers of these animals revealed striking changes. In fistula dogs which succumbed 7 to 10 days after the loss of pancreatic juice, early degenerative changes such as cloudy swelling and moderate fatty infiltration of the liver cells were observed. In fistula dogs which survived for longer periods (17, 20, 20, 20, 24, 25, 25, 32, 51 and 56 days respectively) the histological alterations were more pronounced and consisted of one or more of the following: fatty infiltration and necrosis of the liver cells, congestion of the capillaries around the central veins, atrophy of the liver cords, dilatation of the bile canaliculi and biliary cirrhosis. Some of the animals in this group received daily intravenous injections of NaCl and $NaHCO_3$; others continued for 3 weeks or longer without any form of treatment. Extensive fatty infiltration of the liver also occurred after prolonged obstruction of the pancreatic ducts (80 days).

After varying periods of exclusion of pancreatic secretion from the intestine, the dogs lost their appetites, declined rapidly in weight, showed marked weakness and apathy, became jaundiced in a few instances, developed a bloody diarrhea and finally succumbed. Vomiting did not occur except in isolated instances.

Allan, Bowie, MacLeod and Robinson[3] observed similar symptoms and pathologic changes, in depancreatized dogs treated with insulin. They found an actual increase in the total lipids of the liver and suggested that the pancreas produced an internal secretion, possibly a lipase, which was required by the liver for the mobilization of fat. Fisher[4] noted that the livers of depancreatized dogs kept alive with insulin were fatty. Pflüger[5] had found that the fat content of the livers of dogs was greatly increased after subtotal pancreatectomy and ligation of the pancreatic ducts. Microscopic examination revealed fatty infiltration of the liver cells in the periphery of the lobules and an accumulation of brown pigment in the cells in the central areas. Lombroso[6] concluded from his experiments that the pancreas contained an internal as well as an external secretion which played a rôle in the assimilation of fat. Lombroso also noted that dogs with pancreatic fistulas made according to Pawlow's technique

[3] Allan, F. N., Bowie, D. J., MacLeod, J. J. R., and Robinson, W. L., *Brit. J. Exp. Path.*, 1924, **5**, 75.

[4] Fisher, N. F., *Am. J. Physiol.*, 1924, **67**, 634.

[5] Pflüger, E., *Arch. f. d. ges. Physiol.*, 1905, **108**, 115.

[6] Lombroso, U., *Arch. f. d. ges. Physiol.*, 1906, **112**, 531; *Arch. f. exp. Path. u. Pharm.*, 1908, **60**, 99.

never survived longer than 3 months if the minor duct was ligated at the time of the establishment of the fistula. In addition he observed that many animals began to show signs of extreme weakness and marasmus 30 to 40 days after ligation of both pancreatic ducts and died shortly afterward. He was unable to explain the cause of death of these animals. Recently Hershey and Soskin,[7] continuing the work of Allan, Bowie, MacLeod and Robinson came to the conclusion that the liver was the seat of the major disturbances following pancreatectomy in dogs and that the symptoms were attributable to "liver failure". They believed that the disturbance in the liver was concerned principally with faulty fat metabolism. The addition of "lecithin" to the diets of their animals alleviated the symptoms and improved the function of the liver.

Since our studies show that the symptoms and liver changes following the drainage of pancreatic juice by a fistula or after ligation of the pancreatic ducts are practically identical with those following pancreatectomy, it seems likely that the underlying factor common to the 3 conditions is the absence of the external secretion from the intestine. Our observations indicate that the pancreatic juice contains a substance, possibly hormonal in nature, which is reabsorbed from the intestinal tract and is concerned with fat metabolism in the liver. The marked fatty infiltration which occurs in the liver[8] after the diversion of the portal blood by an Eck fistula may also be associated with a prolonged deficiency in this factor. The liver changes which are found after prolonged inanition resemble those which develop after the deprivation of pancreatic juice. We are undoubtedly dealing with a rather complex phenomenon and at this time merely wish to report our findings to date. It is apparent that a deficit of electrolytes[9] is by no means the only untoward result following the drainage of pancreatic secretion through a fistula.

[7] Hershey, J. M., and Soskin, S., *Am. J. Physiol.*, 1931, **98**, 74.

[8] Berg, B. N., Cone, W. V., and Jobling, J. W., PROC. SOC. EXP. BIOL. AND MED., 1925, **23**, 81.

[9] Gamble, J. L., and McIver, M. A., *J. Exp. Med.*, 1928, **48**, 859.

5729

Local Cerebral Anaphylaxis in the Dog.

LEO M. DAVIDOFF AND NICHOLAS KOPELOFF.

From the Department of Bacteriology, Psychiatric Institute, New York.

The possible rôle of allergy in the mechanism underlying certain neurological conditions offers an interesting field of investigation. Following the recent demonstration by David and Beatrice Seegal of local organ hypersensitivity in the anterior chamber of the rabbit eye, one of us (L.M.D.) in collaboration with the Seegals produced the same phenomenon in the brain of rabbits. However, the sterile inflammation was observed only after making a second injection directly into the brain, and not, as in the case of the eye, by a simple intravenous injection. It occurred to us that this difference might be accounted for by the fact that in the eye there was more prolonged and more intimate contact of antigen with local tissue in a confined space than in the brain. How to effect this in the brain was our immediate problem.

The following procedure was carried out, using dogs as experimental animals:

Under local anesthesia a trephine hole was made and somewhat enlarged by rongeurs over the left motor area. The dura was then opened, and into a cavity in the brain tissue there was inserted a 0.5 cm. cube containing normal horse serum to which enough agar was added to make a jelly (0.6%). The wound was then closed. Immediately after the operation each dog was given from 1.5 to 5 cc. normal horse serum intravenously.

The brain in each case had been purposely injured on the left side so that immediately after operation and continuing for a few days to a week or 10 days the dogs showed a varying amount of right-sided hemiplegia. In every animal the weakness on the right side had disappeared before the next step in the experiment.

Two to four weeks after the brain operation each dog was given 5 cc. of normal horse serum intravenously. In every case in addition to the local reactions about to be described the animals within a few minutes after the intravenous dose showed some signs of generalized anaphylaxis such as rapid respiration, vomiting, diarrhea and later bloody stools. Out of 5 animals, 4 showed definite, transient, right-sided hemiplegia within a few minutes after the intravenous injection. In 2 animals this reaction was quite marked; in

the 2 others it was slight but definite. One dog failed to show any detectable weakness. The results are summarized in Table I.

TABLE I.

Dog No.	1	2	3	4	5
Date of operation	2-27	2-27	4-9	4-9	4-9
Date of injection	3-8	3-18	5-9	5-9	5-9
Hemiplegia after operation	Yes	Yes	Yes	Yes	Yes
Hemiplegia after injection	No	"	Slight	Slight	"
General reaction	Yes	"	Yes	Yes	"

Other antigens, typhoid bacilli, egg-albumen, etc., are being employed in further experiments involving modifications of technic.

5730

Effect of Adrenalin on the Glucose and Lactic Acid Exchange of the Brain.

L. H. NAHUM AND H. E. HIMWICH.

From the Department of Physiology, Yale University School of Medicine.

It has been shown that the brain removes glucose and lactic acid from the blood stream in both normal and diabetic conditions (McGinty,[1] Himwich and Nahum[2]). Since it is not probable that storage of carbohydrates occurs, the substances thus removed must have an oxidative fate.

Adrenalin in adequate amounts is known to inhibit carbohydrate oxidations (Colwell and Bright,[3] Cori and Cori[4]). It might therefore alter the processes concerned with the removal of glucose and lactic acid. Dogs were used and blood samples were obtained from the superior longitudinal sinus and femoral artery simultaneously. The first experiment consisted of injecting adrenalin ½ cc. every 15 minutes for 6 hours. Blood samples were drawn every hour. Table I presents the results.

When adrenalin was injected in small divided doses in accordance with its well known action, it produced an appreciable increase in the glucose and lactic acid level of the blood. The brain under these

[1] McGinty, D. A., *Am. J. Phys.*, 1929, **88**, 312.

[2] Himwich, H. E., and Nahum, L. H., 1929, **90**, 389.

[3] Colwell, A. R., and Bright, E. M., *Am. J. Phys.*, 1930, **92**, 343.

[4] Cori, C. F., and Cori, G. T., *J. Biol. Chem.*, 1928, **79**, 309.

TABLE I.

Effect of repeated small doses of adrenalin on the glucose and lactic acid of the blood passing through the brain.

Dosage	Time	Glucose			Lactic Acid		
		Arterial	Cerebral	Diff.	Arterial	Cerebral	Diff.
½ cc.	injection						
every	1 hr. later	.131	.088	—43	12	12	0
¼ hour	2 " "	.129	.102	—27	27	18	—9
total	3 " "	.175	.150	—25	36	36	0
11 cc.	4 " "	.172	.120	—52	51	43	—8
	6 " "	.226	.190	—36			
	7 " "	.193	.166	—27	35	50	+15

conditions, however, continued its absorption of glucose from the blood stream throughout the period of the experiment. The lactic acid removal continued during the first 4 hours, following which there was a reversal of the process and from this point on, the brain liberated this substance in appreciable amounts.

TABLE II.

Effect of repeated large doses of adrenalin on the glucose and lactic acid of the blood passing through the brain.

Dosage	Time	Glucose			Lactic Acid		
		Arterial	Cerebral	Diff.	Arterial	Cerebral	Diff.
5 cc	injection						
every	1 hr. later	.093	.076	—17			
½ hour	1 " 35 min. later	.112	.093	—19	44	58	+14
total	2 " 15 " "	.204	.183	—21	58	72	+14
35 cc.	2 " 45 " "	.286	.282	—4	39	51	+12
	3 " 15 " "	.290	.296	+6	69	79	+10

With larger doses of adrenalin both the glucose and lactic acid of the blood rose more rapidly to much higher levels. In the first 3 observations the brain continued to absorb glucose in appreciable amounts. Finally this process ceased when enough adrenalin had been injected to raise the glucose level of the blood to about 290. With regard to lactic acid, it is apparent that throughout the course of this experiment the brain liberated this substance in considerable amounts. In the first 3 observations the glucose removed exceeded the lactic acid liberated. Apparently, not all the glucose absorbed disappeared through glycolysis. It is evident that adrenalin reveals the ability of the brain *in situ* to glycolyse.

5731

Rôle of Hypophysis in the Initiation of Metamorphosis in Bufo.*

BENNET M. ALLEN.

From the University of California at Los Angeles.

The importance of the pars anterior of the hypophysis in the control of the thyroid gland has been clearly shown in many ways and by many investigators. The writer has shown that under normal conditions of iodine content in food and water, amphibian larvae fail to metamorphose in the absence of either or both of these glands. The stage at which development ceases is the same in each instance.

It is natural to inquire which of the two glands takes the leading rôle in initiating the process of thyroid secretion. A crucial experiment consists in making transplants of the hypophysis taken from tadpoles of different stages of development into a uniform stage shortly before the beginning of metamorphosis. The recipients of these transplants were selected tadpoles of *Bufo halophilus* with a total length of 25-28 mm. and a hind limb length of 1-2 mm. At the end of from 10 to 18 days they were killed and preserved for study.

The transplants were placed in pockets under the skin median and caudal to the right eye where they could be seen during the course of the experiment. At the end of the experiment they were removed and studied in order to note their condition. Certain of these transplants were sectioned and studied histologically in order

TABLE I.

Donors	Number Cases	Hind Limb Length of Recipients at End of Experiment
		mm.
1. Hind limbs, 2 mm. length. (Two glands transplanted)	30	2.10
2. Hind limbs, 5-8 mm. length. (One gland transplanted)	8	2.34
3. Hind limbs, 8-9 mm. length. (One gland transplanted)	15	3.85
4. Hind limbs, 10 mm. length. (One gland transplanted)	18	5.93
5. Metamorphosis completed.	22	4.93
6. Adult. (¼ pars anterior of hypophysis used)	10	5.69
7. Controls	14	2.28

* This work was carried on with the assistance of research grants of the University of California and of the National Research Council.

to determine their condition at the end of the experiment. Only those found suitable among the 154 tadpoles were used. These numbered 117, of which 103 received transplants and 14 served as controls.

Table I gives the results. The donor types are indicated at the left while the measure of the development induced is shown by the average hind limb length of the recipients indicated.

The duration of the experiment, 10 to 18 days, has not permitted complete metamorphosis but the tendency is quite clear as shown by the length of hind limb and is far more striking as observed in the less measurable changes of body form. The tadpoles of groups 3 to 6 have the characteristic signs of metamorphosis as bulging eyes, laterally compressed angular bodies and shortened tails.

The experiment is being repeated upon a larger scale and under more uniform conditions of date and duration of experiment in order to determine whether the hypophysis is truly more active when the donor has a hind limb length of 10 mm. than at the later period of metamorphosis and even of adult life. The point is an interesting and significant one because of the fact that this is just at the crucial period at which metamorphosis becomes most active. Hypophysectomized and thyroidectomized tadpoles of Bufo attain a limb length of but little less than this. Our results show that the transplants taken from donors of this stage exert a far greater effect than those taken from younger donors. Transplants from tadpole donors of 2 mm. hind limb length do not induce any tendency to metamorphosis even when transplanted in double dose and in experiments that ran the same length of time (15 days) as that of the 10 mm. hind limb donors that gave the maximum effect.

It is clear that the hypophysis plays a major rôle in initiating the activity of the thyroid gland which comes into play only when the hypophysis reaches a certain stage in its development. These facts, taken in connection with histological studies that we have under way, should give us knowledge regarding the type of cells in the pars anterior of the hypophysis that are concerned with the thyroid function.

5732

A New Method for Testing the Potency of Antineuritic Concentrates.

W. FREUDENBERG AND L. R. CERECEDO. (Introduced by C. L. A. Schmidt.)

From the Division of Biochemistry, University of California Medical School, Berkeley.

Experiments undertaken to isolate the antineuritic vitamin, made it necessary to choose a method for testing the potency of the antineuritic concentrates obtained in the course of our work. Although the procedures which use pigeons and rats have been well standardized, we thought it worth while to use mice as test animals. We reasoned that the mouse, on account of its high metabolic rate and its small size, would offer certain advantages. Much less material would be necessary for making the tests and we expected that mice would show symptoms of Vitamin B deficiency at an earlier date than other species.

The results have fulfilled our expectations.

For the preparation of the antineuritic concentrates we followed in general the method described by Jansen and Donath,[1] using the modifications recommended by Jansen.[2] We have been able to isolate a substance which in form of the (impure) hydrochloride is curative in doses of less than 0.025 mg. per day. Jansen and Donath[3] showed that their preparation at this stage of the isolation process was potent for ricebirds in daily doses of 0.008 mg.

We have tested the potency of our antineuritic concentrates at 3 stages of the isolation process. For the first test 6 animals and 2 controls were used, for the second test, 5 animals and 2 controls, for the third test, 6 animals and 3 controls.

Our experiments show that mice may be used with advantage for testing the potency of antineuritic concentrates. Our results corroborate the findings of Jansen and Donath with still another species, the mouse.

[1] Jansen, B. C. P., and Donath, W. F., *Proc. Akad. Wetenschappen Amsterdam*, 1926, **29**, 10.

[2] Jansen, B. C. P., *Rec. Trav. Chim. Pays-Bas*, 1929, **48**, 984.

[3] Local citation.

5733

Studies on the Intermediary Metabolism of Purines and Pyrimidines.

LEOPOLD R. CERECEDO. (Introduced by C. L. A. Schmidt.)

From the Division of Biochemistry, University of California Medical School, Berkeley.

Previous experiments[1] indicated that in the metabolism of uracil in the dog we are dealing with the following sequence of reactions: Uracil → Isobarbituric Acid → Isodialuric Acid → Urea + an unknown carbon compound. Offe[2] has shown that formyloxaluric and oxaluric acids are intermediate products in the oxidation of isobarbituric acid *in vitro* by means of permanganate. Quite recently, Johnson and Flint[3] have found that these compounds are also formed in the course of the oxidation of uracil by ozone.

Formyloxaluric acid and oxaluric acid were fed to dogs maintained in nitrogen equilibrium. In every case there was an increase in the urea output, suggesting that these substances were metabolized. Injection experiments with oxaluric acid yielded the same results. Formyloxaluric acid, on the other hand, when injected, was found to be toxic.

These findings seem to indicate that in the metabolism of uracil in the dog we are dealing with an oxidation resembling that *in vitro* as follows:

Uracil → Isobarbituric Acid → Oxaluric Acid → Urea + Oxalic Acid.

They also suggest that oxaluric acid might be an intermediate product in purine metabolism. The formation of this substance as an end-product of the oxidation of uric acid by various oxidizing agents is a well-known fact.[4]

Experiments were also carried out with alloxan and parabanic acid to determine whether these substances might be intermediary products of purine breakdown in the animal body. Parabanic acid, when fed to dogs in nitrogen equilibrium, is excreted unchanged. The compound was isolated from the urine as the dixanthydryl derivative.

Feeding experiments with alloxan show that this substance is

[1] Cerecedo, L. R., *J. Biol. Chem.*, 1930, **88**, 695.

[2] Offe, G., *Ann. Chem.*, 1907, **353**, 278.

[3] Johnson, T. B., and Flint, R. B., *J. Am. Chem. Soc.*, 1931, **53**, 1077.

[4] Biltz, H., and Schauder, H., *J. f. prakt. Chem. N. F.*, 1923, **106**, 108.

excreted only to a small extent in the urine; the greater part is apparently excreted in the bile. After feeding alloxan we observed a distinct decrease in the output of inorganic sulfur in the urine.

5734

On the Isolation of Guanidine Compounds from the Urine.

M. STOCKHOLM AND L. R. CERECEDO. (Introduced by C. L. A. Schmidt.)

From the Division of Biochemistry, University of California Medical School, Berkeley.

In an attempt to find a reagent which would enable us to isolate guanidine compounds from urine, experiments were carried out with β-naphthalene-sulfochloride. The substances tested were guanidine, methylguanidine, as-dimethylguanidine and synthalin (diguanidinodecamethylene). We have found that all these compounds react with β-naphthalene-sulfochloride. Except for the methylguanidine derivative, the condensation products are very little soluble in water.

The procedure which we have used to recover the above compounds from the urine is as follows: A certain amount (50-200 mg.) of the substance was added to 50 cc. of human or dog's urine. The urine was evaporated to a volume of less than 10 cc. To the residue water was added, so as to have a total volume of 10 cc. This solution was treated with an equal volume of 4 N-KOH. To this a quantity of β-naphthalene-sulfochloride equivalent to 4 mols, dissolved in 25-35 cc. of ether was added. The mixture was shaken for about 8 hours. The precipitate formed was filtered, washed with water and recrystallized from water.

By means of this method we have been able to recover more than 80% of guanidine and dimethylguanidine added to urine. With methylguanidine the best yield obtained was 66%.

5735

Effects of Certain Pyrimidines on the Sulfur Metabolism of Dogs.

J. A. STEKOL AND L. R. CERECEDO. (Introduced by Carl L. A. Schmidt.)

From the Division of Biochemistry, University of California Medical School, Berkeley.

It was observed by Cerecedo[1] that after feeding isobarbituric acid to dogs there was a distinct decrease in the urinary output of inorganic sulfates and a corresponding rise in the ethereal sulfur fraction. These findings led him to assume that isobarbituric acid was partly excreted in the urine in conjugation with sulfuric acid, as an ethereal sulfate.

The present investigation represents an extension of the previous work. After feeding isobarbituric acid in doses of 2.5 gm. to dogs, which were maintained on a nitrogen equilibrium, we found that 60-80% of the substance was metabolized to urea, 20-25% excreted as ethereal sulfate, and the remainder eliminated unchanged.

Very striking is the effect of the ingestion of isobarbituric acid on the neutral sulfur fraction in the urine. We observed that after the feeding of the substance there was no detectable amount of neutral sulfur excreted. The disappearance of the neutral sulfur was also observed after the ingestion of isodialuric acid. These observations lead us to assume that in the catabolism of these compounds a sulfur containing substance is involved, which is normally present in the neutral sulfur fraction of the urine.

From the urine of dogs which had ingested isobarbituric acid, we have been able to isolate a compound in form of its xanthydrol derivative. The analytical results so far obtained indicate that we are dealing with dixanthydryl isobarbituric acid sulfate.

[1] Cerecedo, L. R., *J. Biol. Chem.*, 1930, **88**, 695.

5736

Ovulation in the Neotenic Amblystoma Tigrinum Following Administration of Extract of Mammalian Anterior Hypophysis.

ADRIAN BUYSE AND R. K. BURNS, JR.

From the Anatomical Laboratory, University of Rochester School of Medicine and Dentistry.

Ovulation in adult amphibians has been experimentally induced by hypophyseal transplants or by administration of extracts of the hypophysis in a number of species by various workers. Wolf[1] succeeded in producing ovulation out of season in frogs by means of homoplastic transplantation of anterior hypophysis. Houssay, Giusti, and Lascano-Gonzalez[2] and Houssay and Giusti[3] were able to bring about a similar response in toads by the use of homoplastic implantations of anterior lobe. The results obtained by Noble and Richards[4] on the salamander *Eurycea bislineata,* in which ovulation has been induced by means of homoplastic transplants, are confirmed by the findings of Adams[5, 6] who was able to obtain egg-laying in adult *Triturus viridescens* and *Triton cristatus* by the same means. Similar results were observed by Adams[7, 8] if heteroplastic transplants from anurans were substituted for the homoplastic implants. Adams[9] was also able to confirm the earlier findings of Houssay *et al* on ovulation in toads, in being able to elicit the response only with homoplastic transplants of anterior lobe substance.

Ovulation has likewise been produced in anuran amphibians with extracts of the mammalian hypophysis. Through the administration of an alkaline aqueous extract of the anterior lobe of cattle, Kehl[10] induced ovulation in the mature frog, *Discoglossus pictus,* and Adams[9] using a similar extract obtained the same response in frogs. Up to the present time there appears to be no record of

[1] Wolf, O. P., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 692.

[2] Houssay, B. A., Giusti, L., Lascano-Gonzalez, J. M., *Rev. Soc. Argent. Biol.*, 1929, **5**, 397.

[3] Houssay, B. A., and Giusti, L., *Compt. Rend. Soc. Biol.*, 1930, **104**, 1030.

[4] Noble, G. K., and Richards, L. B., *Am. Mus. Novitates,* No. 396. 1930.

[5] Adams, A. E., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 433.

[6] Adams, A. E., *Anat. Rec.*, 1930, **45**, 250.

[7] Adams, A. E., *Anat. Rec.*, 1931, **48**, 37.

[8] Adams, A. E., *Anat. Rec.*, 1931, **48**, 38.

[9] Adams, A. E., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 677.

[10] Kehl, R., *Compt. Rend. Soc. Biol.*, 1930, **103**, 744.

ovulation in urodele amphibians produced by means of extracts of mammalian hypophysis.

Experiments on the induction of ovulation were carried on during the month of June with 2 adult female Axolotls (usually considered a neotenic form of *Amblystoma tigrinum*) known not to have ovulated previously in the laboratory. These animals were given daily 1 cc. of extract of whole sheep's pituitary gland* injected into the peritoneal cavity. This extract had already proved active in producing accelerated growth and premature activity in the immature gonads of larval salamanders.[11]

At the end of the second day one animal gave a positive response to the treatment by spawning several dozen eggs. The second animal did not respond until the third day when 40 to 50 eggs were laid. A few hours after administering the third cc. of the extract to the first animal a copious spawning occurred, approximately 500 ova being liberated. The response of the second animal could not be increased by further administration of the extract, so the experiment was discontinued for the time.

On the arrival of a new shipment of extract prepared in March, and of proved potency in stimulating the gonads of immature female rats and dogs, it was decided to repeat the experiment on the second animal. Thirteen days having elapsed since the previous injection, 1 cc. of the new extract was given intraperitoneally consecutively for 4 days. On the fourth day approximately 2 dozen ova were extruded. In addition to the extrusion of ova, numerous small masses of caseous debris, imbedded in strings of gelatinous material, were also found with the spawned ova in the container. The debris was found to be clumps of resorbing ova which were doubtless ovulated during the previous administration of the extract, but not extruded from the abdominal cavity.

* Alkaline aqueous extract of whole sheep's pituitary as prepared by Parke Davis & Co., and kindly supplied to us through the courtesy of Dr. E. P. Bugbee. For details of preparation refer to Bugbee, Simond and Grimes, *Endocrinology*, 1931, **15**, 41.

[11] Burns, R. K., Jr., and Buyse, Adrian, *Anat. Rec.*, 1931, **48**, 12.

5737

Developmental Aspects of the Electrocardiogram.

MAURICE DIONNE, ELWOOD SCHAFER AND HERBERT POLLACK.
(Introduced by Shields Warren.)

From the Laboratory of Pathology, New England Deaconess Hospital, and the Lahey Clinic, Boston.

Recent improvements in the technique previously described[1, 2] have been made by the addition of a special circuit[3] with 3 vacuum tubes between the electrodes and the galvanometer. This apparatus gives a known and desired amplification (5 to 30 times) of the heart voltage, without distortion. By its use, a new series of electrocardiograms of the chick embryo from the fourth day to the twentieth day was taken.

Five distinct waves are seen as early as the fourth day. These, like those of the adult mammalian heart, can be separated into 2 parts, the auricular complex and the ventricular complex. The auricular complex consists of the P-wave which is followed immediately by a wave of longer duration, Ta, which occupies the remainder of the P-R interval. The analogy may be drawn between this new wave (Ta) and the T-wave following the ventricular complex. The ventricular complex starts with a sharp summit R, and a dip S. There is no Q-wave in any record. The T-wave follows, always diphasic in type. The recorded voltages on the fourth day were read from the β lead and the circuit resistance was 4300 to 5400 ohms.

The average P-wave deflection was 0.015 millivolts, Ta-wave was 0.010 millivolts. The average R-wave was 0.070 millivolts, the S-wave 0.070 millivolts, and the T-wave took origin 0.017 millivolts negatively to the isoelectric and after becoming 0.018 millivolts negative went to a summit of 0.003 millivolts before returning to the base line.

In the α lead the P-wave is diphasic up to the tenth day inclusive, after which it is always negative. The Ta wave is positive to the eleventh day inclusive and then becomes negative. The R-wave is upright, the S-wave dips, and the T-wave always starts negatively to the isoelectric line and is invariably diphasic.

---

[1] Pollack, H., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 61.

[2] Pollack, H., *J. Lab. and Clin. Med.*, 1931, **16**, 1194.

[3] Pollack, H., Dionne, M., and Schafer, E., *J. Lab. and Clin. Med.*, 1931, **16**, 1198.

The β lead, as well as the gamma lead, is very similar in form to the α, except that the P-wave is always positive.

The deflections are low at first, come to their greatest height at the eighth to ninth days in the auricular complex, and at the ninth to tenth day in the ventricular complex. Following these dates, they again become low to gain slowly afterwards to the twentieth day.

The behavior of these tracings points to a possible relation to the establishment and initiation of a nervous control over the chick embryo heart, which occurs at approximately the same time. Thus, nervous control would be inhibitory in type and would affect the auricular complex earlier than the ventricular complex. The series is now being extended to include the first days of life outside the shell.

5738

Pellagra and Vitamin Deficiency.

TOM DOUGLAS SPIES. (Introduced by J. T. Wearn.)

From the Medical Clinic of Lakeside Hospital and the Department of Medicine, Western Reserve University.

Despite the fact that pellagra is a definite clinical entity, extensive investigation over several centuries has failed to establish a causative agent.[1, 2, 3] Of all the many theories advanced as to its etiology, the two most important would seem to be infection or dietary deficiency. All attempts to transmit the disease have failed and no incontrovertible evidence of an infectious agent have been advanced. The relationship of this disease to poverty has been stressed since its earliest recognition. Goldberger, *et al*,[3, 4, 5, 6] brought forward considerable additional evidence toward establishing pellagra as a dietary deficiency disease.

---

[1] Wood, Edward Jenner, "Pellagra." Oxford Medicine, Vol. IV, part L, page 307.

[2] Funk, C., *Die Vitamine.* Wiesbaden, 1914.

[3] Goldberger, J., "The transmissibility of pellagra." *U. S. Public Health Rep.*, Nov. 17, 1916, page 3159.

[4] Goldberger, Waring, and Willets, "The prevention of pellagra." *U. S. Public Health Rep.*, 1915, XXX, page 3116.

[5] Goldberger, Wheeler, and Sydenstricker, "The relation of diet to pellagra incidence." *U. S. Public Health Rep.*, March 19, 1920, XXXV, page 648.

[6] Goldberger, J., and Wheeler, G. A., "Experimental production of pellagra in human beings by means of diet." Hygienic Laboratory, Washington; Bull. 120, Feb. 7, 1920.

There has been considerable difference of opinion as to the relationship of chronic alcoholic intoxication to this disease. It is certain that pellagra often occurs without alcoholism. On the other hand, it seems fair to admit that sufficient indulgence in alcohol may prevent an adequate food intake, thus favoring the production of pellagra.

During the past several decades innumerable minerals, drugs, and diets have been proposed as a cure for this disease. Controversy has inevitably arisen as to their efficacy. In view of the large number of inadequately controlled experiments, it seemed especially worthwhile to study the acute phase of this disease under strict experimental conditions.

The present report deals with the study of four cases of pellagra which were limited to a diet deficient in minerals and vitamins "B" and "C".

Three of the four classical cases of pellagra chosen were white males with a history of alcoholism. The other case was a negress with no history of drinking during the past few years. All the patients had a definite history of dietary deficiency extending over a period of two months or longer. Three patients had diarrhea; three had stomatitis, three had anal lesions, two had mild mental deterioration; the one female had vaginal ulcerations. The four patients had characteristic bilateral symmetrical dermatitis of the hands. In addition, the negress had a butterfly-shaped, symmetrical lesion on the vulva, thighs, and anus, typical of pellagra.

Each patient received a daily diet of 2,300 calories (cal. from C = 1700, cal, from P = 110, cal. from F = 490) consisting of: corn meal mush, corn meal muffins, pork fat, maple syrup, polished rice (boiled), cornstarch pudding, coffee, and sugar. This diet was administered from the time the patient entered the hospital until the day prior to discharge when he was given a high protein, high vitamin diet.

It can readily be seen that the diet used was even more restricted in mineral content and in vitamin "C" and "B" (including pellagra-preventive factor) than Goldberger's pellagra-producing diet.[6] (He produced pellagrous lesions of the scrotum in six of eleven normal subjects after five months.)

The condition of all patients rapidly improved (see Figs. 1 and 2). The stomatitis, diarrhea, and anal lesions were relieved during the first three hospital days. The deep purplish erythema over the hands and tongues of the three white male patients disappeared during the first week. Desquamation of the involved epithelium

FIG. 1.
Showing the symmetrical lesion of pellagra.

then began in the central portions and progressed toward the periphery. The involved sites became covered by thin soft epithelium of pink color during the second hospital week. The skin lesions of the negress improved less rapidly than those of the white males and desquamation did not appear before the end of the second week. During the third and fourth weeks the thick, roughened skin was then replaced by soft, deeply pigmented epithelium.

The mental change showed much less change during the period of observation.

Conclusions. Four cases of moderately severe pellagra have been presented, which improved strikingly on a diet of 2,300 calories de-

FIG. 2.
The same patient 23 days later. Note, the chief residual change is due to freckles.

ficient in mineral content and vitamins "C" and "B" (including pellagra-preventive factor). The patients showed no return of signs or symptoms during the six to seven weeks they received this diet.

5739

A Chemical Factor in the Causation of Tingling Sensations During and after Release of Stasis.

H. C. BAZETT AND B. MCGLONE.

From the Physiologic Laboratory, University of Pennsylvania.

At the XIIIth International Physiological Congress[1] a report was made of experiments on the effect of temperature on sensations caused by stasis and its release. Attention was directed entirely to sensations of temperature, but observations had also been made on tingling sensations, and the effects observed have been discussed with many workers. Since the detailed report of these experiments has been delayed in publication, it is desirable that a brief summary of the observations be communicated at this time.

Stasis was produced by a Riva Rocci armlet above the elbow, or by a similar compressing band at the base of one finger. The tissues submitted to stasis were cooled and warmed before, during, and after compression by immersion in rapidly stirred water at a constant temperature to allow thermal equilibrium to be attained. Immersion was continued for at least 20 minutes prior to induction of stasis.

The temperature of the peripheral part of the arm was found to modify profoundly the development of tingling sensations. Stasis to the arm for 5 minutes immersed in water at 24° gave practically no tingling sensation, but, if the forearm was immersed in water at 39°, tingling sensations were intense with the same duration of stasis. At still higher temperatures the tingling developed more rapidly, and very early became altered into a more intense, more continuous, less bearable sensation of somewhat different type. The subject interpreted the sensation as having an element of internal tension. Such sensations have been previously explained as due to pressure on the nerve trunks, but in our experiments no considerable alterations in the condition of these nerves above the water level can be assumed. The causation of the sensations must be peripheral.

During stasis tingling, once developed, remained in evidence but might fluctuate in intensity. On release of stasis it immediately disappeared but returned ½ to 1 minute later in a much more intense form. After prolonged stasis the tingling might last many minutes. The tingling both during stasis and on release was much

[1] *Am. J. Physiol.*, 1929, **90**, 278.

more in evidence the longer the period of stasis, and the higher the temperature of the peripheral surface. Short periods of stasis (2½ to 5 minutes) gave no tingling at the lower, but intense sensations at the higher temperatures.

Stasis of the finger alone even at the higher temperatures (*e. g.*, 39° for 5 minutes) gave only very slight tingling during stasis and on release, while if the compression was applied to the arm, the fingers appeared to share in the tingling sensations. In the fingers the nerve trunks must be peculiarly exposed to mechanical effects of pressure, and in this locality the changes in temperature also involved these nerve trunks.

It may, therefore, be concluded that the sensations of tingling are induced, directly or indirectly, by some chemical factor, the rate of formation of which varies with the temperature of the tissues. This factor presumably is formed more rapidly in the muscles, since stasis involving muscular regions is much more effective than stasis of the finger, which contains only smooth muscle.

A chemical factor seems the most legitimate hypothesis, but it might act indirectly through inducing osmotic changes.

It should be noted that the tingling was not investigated in detail, since the primary object of the experiments was to study temperature sensations and to arrange the experiments in such a way that tingling did not offer too great a complication.

5740

Carotid Sinus Reflexes to the Respiratory Center.

CARL F. SCHMIDT.

From the Laboratory of Pharmacology, University of Pennsylvania.

According to Heymans[1] and Koch,[2, 3] changes in pressure within the carotid sinuses set up reflexes which have a powerful influence upon the respiratory center. Their observations are fully confirmed by the results of perfusion of the carotid sinuses of 15 dogs, 5 rabbits, and 4 cats, with Locke's solution or with blood from a donor animal, by means of a pump. Heymans'[1] conclusion, that

[1] Heymans, C., and Bouckaert, J. J., *J. Physiol.*, 1930, **69**, 254.

[2] Koch, E., *Die reflektorische Selbststeuerung des Kreislaufes.* Dresden, 1931, 168.

[3] Koch, E., and Mark, R. E., *Z. f. Kreislaufforsch.*, 1931, **23**, 319.

the respiratory stimulant effect of occlusion of both common carotids is entirely the result of carotid sinus reflexes, is not confirmed; denervation of the carotid sinuses, while usually diminishing and sometimes abolishing the stimulant effect upon breathing, occasionally does not decrease or may actually increase the respiratory effect of the occlusion, in decerebrate cats and narcotized dogs; hypertension as a result of the denervation introduces a new factor in that carotid occlusion does not reduce cerebral blood-flow to as low a level as before; a negative result from the occlusion does not then prove that a previous positive result was entirely due to reflexes from the carotid sinuses. Carotid occlusion lowers endosinusal pressure by 36% (average) in dogs; it reduces cerebral (torcular) venous outflow by about 45% (average) in dogs. Occlusion of the vertebral arteries reduces torcular outflow only by 25% (average) in dogs, and in one animal it had no measurable effect; absence of circulatory and respiratory stimulation from occlusion of the vertebrals does not therefore necessitate the conclusion that the stimulant effects of carotid occlusion are due entirely to reflexes from the carotid sinuses.

Heymans' conclusion[1] that the respiratory center is not directly affected by increase in its blood-supply is contradicted by the results of 4 sets of experiments in which medullary blood-flow was increased under such circumstances that reflexes from the carotid sinuses or aorta could not contribute to the results:

1. Reopening of occluded carotid and vertebral arteries produces apnea in dogs and cats. The result is exactly the same whether the occlusion is made proximal (common carotids) or distal (external and internal carotids) to the carotid sinuses; it is not significantly altered by sinus denervation. The apnea is not prevented by continuous inhalation of 10% CO_2, so that it is not due to the hyperpnea resulting from the occlusion.

2. Lowering of intracranial pressure to zero after a brief period of elevation to a level close to that of arterial blood-pressure (by way of a parietal cannula or cisternal needle) produces respiratory depression or apnea. Section of the sinus and aortic nerves does not modify the result. CO_2 inhalation shortens the apnea but does not prevent respiratory depression upon reduction of extramedullary pressure.

3. Adrenalin frequently produces apnea in dogs and cats, occasionally in rabbits, whose aortic and sinus nerves are completely

divided, or whose carotids (in sinus perfusion experiments) are isolated from the systemic circulation with aortic nerves cut.

4. Increase in vertebral perfusion flow was found[4] to cause respiratory depression in dogs and cats; in those experiments the common carotids were closed, so that sinus reflexes were presumably excluded, and aortic reflexes operated in the reverse direction because increase in perfusion flow necessitated decrease in systemic blood-pressure. Recently the brains of 5 rabbits have been similarly perfused by way of the internal carotids, with sinus and aortic nerves cut; in these also respiration was regularly depressed by an increase in cerebral blood-flow, stimulated by a decrease (*e. g.*, increase in flow from 25.6 to 40 cc. per minute reduced breathing from 1280 to 640 cc. per minute; decrease in flow from 42.8 to 26.3 cc. increased breathing from 432 to 800 cc. per minute).

The experiments of Heymans, Bouckaert, and Dautrebande[5] dealing with carotid sinus reflexes aroused by changes in gas-content of the carotid blood have been repeated. Of 10 dogs, only one showed repeatable and reversible effects of this sort and this one was least sensitive of all to the reflex respiratory effects of changes in carotid sinus pressure. Of 4 cats, however, all showed definite effects of the sort described by Heymans *et al.* In every case the possibility of patent carotid branches in the recipient animal was excluded. The influence of sinus denervation upon the respiratory effects of repeated exposure to CO_2 excess and oxygen-lack (nitrogen inhalation) was investigated in 4 dogs, one cat, and one rabbit. In general, the stimulant effect of either gas was less after sinus denervation than before, and that of oxygen-lack was reduced more than that of CO_2 excess. Sometimes anoxemia failed entirely to stimulate breathing after sinus denervation (one out of 7 inhalations in the cat, 4 out of 10 inhalations in the dogs), but in no case was this result obtained consistently in the same animal; other inhalations of the same gas-mixture by the same animal sometimes stimulated breathing more than they had before sinus denervation. In no case did CO_2 (10% in oxygen) fail to stimulate breathing after sinus denervation. These results are difficult to interpret; they indicate that while the sinus nerves apparently have some relation to the respiratory effects of asphyxia and anoxemia, the relation is not the simple one outlined by Heymans.[5]

It is concluded that while changes in carotid pressure undoubt-

[4] Schmidt, C. F., *Am. J. Physiol.*, 1928, **84**, 202.

[5] Heymans, C., Bouckaert, J. J., and Dautrebande, L., *Arch. internat. de Pharm. et de Therap.*, 1930, **39**, 400.

edly affect respiration reflexly through the sinus nerves, changes in blood-supply of the center can produce identical results. No qualitative differences have been disclosed; both influence depth of breathing more regularly and more readily than rate; both are effective only for a brief time after existing conditions of carotid pressure or cerebral flow have been changed, respiration returning toward normal when pressure or flow is maintained at a new level; both are rendered more effective by a preliminary period of subnormal carotid pressure or cerebral flow. The importance of carotid sinus (and aortic) reflexes in the respiratory responses of the intact animal to changes in blood-pressure can be determined only by quantitative data concerning relative intensities, relative sensitivities, latent periods, effective ranges, etc., of the reflexes on one hand, of alterations in blood-supply of the center on the other. The significance of sinus reflexes to the chemical regulation of breathing is more complicated than was supposed by Heymans;[5] in all probability factors are involved that have not yet been identified. Evidence now at hand suggests that sinus reflexes affect the respiratory center not directly, but through changes in caliber of the finer blood vessels supplying it. Experiments intended to yield quantitative data are being made.

5741

A Method of Assay of Extracts Containing the Suprarenal Cortical Hormone.

R. L. KUTZ. (Introduced by J. B. Collip.)

From the Department of Biochemistry, McGill University, Montreal.

This investigation was begun with the object of developing a method for the assay of extracts containing the cortical hormone which could be carried out with smaller amounts of extract than is required when bilateral adrenalectomized cats or dogs are used.

Hartman[1] has used the growth curve of treated suprarenalectomized rats of 100 to 150 gm. in weight as an alternative method of assay.

It was discovered that immature rats of the colony in this laboratory (Wistar strain) which has been inbred by us for a number of

[1] Hartman, F. A., and Thorn, G. W., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 94.

years do not survive suprarenalectomy, except in rare instances. For example, of a group of 57 from which the glands were removed at 4 weeks of age, 56 had died on the tenth day. A search of the literature did not reveal any data indicating the ability of such young rats to survive bilateral suprarenalectomy and there has been little agreement between the results of various authors concerning the survival period of suprarenalectomized adults. It seemed advisable, therefore, to investigate the possibility of using such animals for the standardization of potent extracts of the cortical hormone. The results of this study are most encouraging, and it is suggested that a rat unit be taken as the minimum daily dose of extract which will protect (for at least 20 days) 50% of a group of animals suprarenalectomized at 28 days of age, the extract being administered subcutaneously twice daily. Table I illustrates an experiment in which the potency of an extract has been demonstrated.

TABLE I. *Assay of Extract.*

Rats Used	Daily Dose. Equiv. in gm. of original glands	Rats not surviving 20 days	Remarks
	gm.		
2	15	0	Normal growth rate after recovery from operation.
2	10	0	Normal growth rate after recovery from operation.
2	5	0	Slightly less than normal growth rate after recovery.
5	2	3	Reduced growth rate in survivors.
2	1.5	2	
2	1.0	2	
2	0.5	2	

The extracts used at first were made according to Swingle and Pfiffner's[2] method. Later it was found that the following simplified process was satisfactory.

The whole glands (beef) were first extracted with an equal volume of absolute acetone. The acetone extract was concentrated at low temperature and pressure to one-thirtieth of its original volume. The concentrate was extracted 5 times with benzene. The combined benzene extracts were reduced in volume at low temperature and pressure and subsequently washed with 4% sodium bicarbonate solution until all adrenalin was removed. After further concentration, the benzene extract was converted into a water extract as follows: An equal volume of water was added to the benzene solution

[2] Swingle and Pfiffner, *Am. J. Physiol.*, 1931, **96**, 164, 180.

and the benzene was removed by distillation. Acetic acid was added to the watery extract until maximum precipitation of lipoid was obtained. The precipitate of lipoid was removed by filtration. The filtrate was clear and almost colorless. It was made to represent 15 gm. of whole glands per cc.

5742

Influence of Laughter on Muscle Tone.

HARRY A. PASKIND. (Introduced by L. J. Pollock.)

From the Department of Nervous and Mental Diseases, Northwestern University Medical School, Chicago, Ill.

This study had its inception in the observation that in narcolepsy there occur attacks of cataplexy or tonelessness on emotion, especially during laughter and mirth. It was thought worth while to study by objective methods the effect of laughter on muscle tone.

The apparatus used was devised by Dr. L. C. Hutchinson of the University of Minnesota. The method consists essentially of passively flexing the forearm at a constant speed against a flat spring. The greater the resistance offered by the arm, the greater was the deflection of the spring. The spring was connected with a writing lever in such a way that the writing lever rose or fell with increasing or decreasing deflections of the spring. In this way the writer lever writing on a smoked drum described a tracing.

Experiments were done on 50 normal subjects. The first reading was taken with a calm facial expression. Then a reading was taken during laughter. Laughter was induced. In no instance was any considerable amount of mirth present but not all spontaneous laughter is due to mirth. It was thought possible that changes in muscle tone seen on laughter were due to the fact that the subject was distracted by his laughter, for this reason control readings were taken with the subject frowning to rule out the effect of distraction.

It was thought that the best method of measuring variations in muscle tone under the varying conditions of the experiment was to determine the differences in the amount of work done in passively moving the arm a certain distance. Since the area underneath a curve is an indicator of the amount of work done, the measurement of these areas and their comparison afforded a convenient method for the interpretation of the results. The area measured in each tracing was one resembling a right angled triangle. The base was

the base line of the curve. Three inches of base line was chosen arbitrarily because it included all the elements of the curve. The side of this triangle was a line drawn at a right angle to the base line from the base line to the curve. The hypotenuse was the tonus tracing, in all instances irregular. The areas so delimited were measured with a planimeter, which gave readings in square inches. The planimeter readings were taken as an index of the amount of work done and may be called work values. Variations in the work values represent variations in the amount of work necessary to passively flex the arm; they represent variations in the amount of muscle tone. The accompanying table may serve as illustration of the variations in the work values in a few subjects, during repose, during laughter, and during frowning.

TABLE I.

Subject	Flexion of Right Arm. Work Value During		
	Repose	Laughter	Frowning
H. D.	2.33	1.45	2.48
H. C.	3.18	1.03	3.75
D. M.	4.17	2.96	6.34
H. H.	3.43	3.10	4.12
L. R.	4.30	4.07	5.06
C. H.	3.53	2.20	3.53
A. M.	2.13	1.81	2.88
E. R.	3.12	1.89	3.17
K. B.	1.93	1.12	2.85
R. K.	2.60	1.42	2.45

It was found that in 48 subjects, or 96%, there was a diminution of muscle tone during laughter, in 2 subjects, or 4%, there appeared to be an increase in muscle tone during laughter. In 39 subjects, or 78%, there was an increase of muscle tone during frowning; in 8 subjects, or 16%, there was a decrease and in 3 subjects, or 6%, there was no change during frowning.

5743

The Endodermal Origin of Middle Ear Cartilages of Rana.

H. N. VIOLETTE. (Introduced by A. W. Meyer.)

From the Department of Anatomy, Stanford University.

In recent years a small, hitherto unknown cartilage has been described in the Amphibia. This develops at metamorphosis in the

interval between the facial nerve behind and the quadrate cartilage in front. Stadtmüller[1] described a cartilage lateral to the quadrato-mandibular articulation in *Bombinator pachypus* and termed it *"Cart. paraarticularis."* In the same position, in the members of the higher Anura, the *Annulus tympanicus* develops. In 2 urodeles (Litzelmann[2] on Triton and Stadtmüller[3] on Salamandra) a cartilage develops along the posterior border of the quadrate. Litzelmann shows that the tubo-tympanal rudiment of the first visceral pouch of the Triton embryo occurs in this position, but later becomes cut off from the pharynx and disappears. Litzelmann concluded that the cartilage which develops later in this position is the representative of the hyomandibular cartilage of fishes, long sought in the Amphibia.

The tubo-tympanal rudiment of Rana was shown by Spemann[4] to develop from the embryonic first visceral pouch. In the present study, the development of the tubo-tympanum during the transformation of the larva into the adult has been followed. During larval life a collection of mesenchymal cells appears about the distal end of the tubo-tympanal bud. These cells appear to be derived from the epithelium of the bud itself, inasmuch as cells from the latter appear to migrate out into the mesenchyma. The latter condenses, and at transformation, 2 cartilages differentiate in it. One becomes the *Annulus tympanicus*, the other the distal part of the *Columella auris*.

This was established by the following experiments upon a series of *Rana sphenocephala* embryos. A cut was made through the ectoderm along the hyomandibular groove, at a stage in which the external gills I and II each consisted in 2 simple branches. When the edges of the wound were reflected, the extremity of the tubo-tympanal bud was seen bending forward and outward around the hyoid mesoderm. The distal end of the bud was excised by means of glass needles. The individuals of the series were killed at various stages after the operation. At metamorphosis, the tympanic membrane failed to appear on the operated side. Dissection showed that the *Annulus tympanicus* and the distal end of the columella were absent on the operated side. This result bears on the finding of Helff[5] that the formation of the tympanic membrane is depen-

[1] Stadtmüller, F., *Z. f. Anat. u. Entw'gesch*, 1931, **94**, 792.
[2] Litzelmann, E., *Z. f. Anat. u. Entw'gesch.*, 1923, **67**, 457.
[3] Stadtmüller, F., *Z. f. Anat. u. Entw'gesch.*, 1924, **75**, 149.
[4] Spemann, H., *Zool. Jahrb. Abt. Anat. u. Ont.*, 1898, **11**, 389.
[5] Helff, O. M., *Phys. Zool.*, 1928, **1**, 463.

dent upon the cartilaginous *Annulus tympanicus*. The operation produced uniform results in the series. In the larval stages, no trace of the tubo-tympanum was to be seen on the operated side, and the chondroblastemata of the *Annulus tympanicus* and the distal part of the columella failed to appear. In the stages killed during metamorphosis, the tubo-tympanum, the *Annulus tympanicus* and the distal part of the columella were absent. No other abnormalities were evident on the operated side. The proximal part of the columella made its appearance in the *Fenestra vestibuli* as normally, and elongated anteriorly as far as the *Processus ascendens*, which joined the *Crista parotica* as on the unoperated side.

It appears that 2 cartilages in the middle ear of Rana (the *Annulus tympanicus* and the distal part of the columella), are formed from the endoderm of the first visceral pouch. The author wishes to suggest that possibly in urodeles and pelobatids the tubo-tympanal rudiment possesses the capacity to form cartilage, and that the cartilages described by Litzelmann and Stadtmüller are representatives of the chondroblastema in Rana, from which the *Annulus tympanicus* and the distal part of the columella arise.

5744

Glycogen Content of Fresh-Water Mussels.

DEA BAILEY CALVIN. (Introduced by Edgar Allen.)

From the Department of Biological Chemistry, School of Medicine, and the U. S. Bureau of Fisheries Research Laboratories, University of Missouri.

Determinations of the glycogen content of fresh-water mussels have been made, using a modification of the Pflüger method as described by Cori.[1] Separate determinations were made on the hepato-pancreas and foot muscle. The results are expressed in percentages of the wet and dry weights of the tissues analyzed.

Fourteen different species of fresh-water mussels were used in the work. Altogether, 51 mussels were analyzed. Many of them had been in the laboratory for some time. They were kept in tanks containing sand and a steady, slow stream of tap water was allowed to flow over them. No food was given them. Others were brought in for analysis from various sources.

[1] Cori, C. F., 1926, **70**, 559.

A number of the results are interesting. As might be expected, the mussels kept in the laboratory without food showed a gradual decrease in the glycogen content. This seemed to be more pronounced in the hepato-pancreas than in the foot. In *Fusconaia undata,* for instance, the glycogen content (based on wet weight) of the hepato-pancreas dropped from 7.15% on February 14 to 1.24% on April 14, a loss of 82.7%. At the same time the foot content changed from 2.2% to 0.735%, a loss of only 66.6%. Frequently, in fact, in the starved animals, the percentage glycogen content of the foot was found to be slightly higher than that of the hepato-pancreas, while normally in well fed animals the reverse was true, the hepato-pancreas being 2 or 3 times as rich in glycogen as the foot muscle.

In many instances after long continued fasting the mussel contained too little glycogen to be determined accurately. This seems to coincide very well with the results of experiments carried out in this laboratory on foot muscle activity, in which it has been found that in fasting mussels the degree of activity is very low.[2]

In well nourished mussels the glycogen content of the hepato-pancreas is usually 4 to 10% (average = 5.88) of the wet weight and 12 to 35% (average = 27.6) of the dry weight. In one animal, fresh from the river, 61% of the dry weight of the hepato-pancreas was found to be glycogen. For the foot, the usual range is from 1 to 3% (average = 1.91) of the wet weight and 5 to 15% (average = 9.2) of the dry weight.

The solid matter was found to vary between 18 to 28% (average = 21.9) for the hepato-pancreas and 18 to 33% (average = 21.25) for the foot muscle. It will be observed that these are much smaller variations than those found in the glycogen content of the same group of animals.

[2] Ellis, M. M., and Merrick, Amanda D., unpublished results.

5745

Abortive Poliomyelitis.*

S. D. KRAMER AND W. L. AYCOCK. (Introduced by Benjamin Kramer.)

From the Department of Preventive Medicine and Hygiene, Harvard Medical School, and the Research Laboratory, Vermont Department of Public Health.

We have shown a high rate of immunity to poliomyelitis in the general population,[1] and the distribution of this immunity is correlated with the incidence of the disease not only in the different ages but with concentration of population. The vast majority of urban adults were found to be immune and the incidence of immunity decreased rapidly and was lowest in the youngest age groups where the incidence of the disease is highest. We found the same type of curve to obtain in rural communities although there was considerably less immunity present. On comparing our findings with similar findings in diphtheria we found a very close parallelism between the two diseases.

It has become important to determine the mechanism by which this general immunization takes place. Since Wickmann's first suggestion of the probable occurrence of abortive forms of the disease the impression has become general that much, if not all, of this immunity is accomplished through abortive attacks of the disease.

During rather intensive studies in Massachusetts and Vermont over a period of years, we have not been convinced that there are any large numbers of such mild illnesses concurrent with an outbreak of the frank disease. A favorable opportunity to determine the incidence of such abortive forms at the time of an outbreak came to us in October, 1930, when 5 frank cases of poliomyelitis occurred in the town of Bedford, Mass., (population 1700) some 20 miles from Boston. With the appearance of the first case, residence was established in the town, and a house to house canvass made over a period of 6 weeks, and histories of all illnesses within 2 weeks previous to the appearance of the first case were collected. School was in session at the time and all of those stricken with the disease were pupils of the local school. Our previous findings had suggested a widespread dissemination of the virus and it was

* This work was supported by the Harvard Infantile Paralysis Commission, the Vermont Department of Public Health and the International Committee for the Study of Infantile Paralysis.

[1] Aycock, W. Lloyd, and Kramer, S. D., *J. Prev. Med.*, 1930, **4**; *J. Exp. Med.*, 1930, **52**, 457.

hazarded that many of the pupils in the school would be exposed to the virus present in that community at the time. Approximately 50 mild illnesses consisting essentially of unexplained headache, fever and vomiting, lasting about 24 to 48 hours, were found, 33 of these occurring in children under 15 years of age. Every case of such illness occurring at the time of our residence was seen and examined.

To determine whether or not these illnesses were actually abortive forms of poliomyelitis, neutralization tests were done on about half of the children and these were selected to include samples from every age group in that school. In order to properly evaluate the results of these neutralization tests a group of 28 children of the same ages who had not passed through any recognizable illness during that interval of time were taken. A third group of children of the same ages from an adjacent town where no poliomyelitis had occurred were similarly tested. These last 2 groups of children, added to our earlier rural and urban figures, gave a good base line for normal children of these age groups with which to compare the results of the tests upon the sera of those having suspected abortive forms of the disease. The results of the immunity tests in these 3 groups of children proved to be identical. The expectation of a high rate of immunity in those having passed through the mild illness did not materialize. These findings are summarized in the accompanying table. Since these bloods were taken about 5

TABLE I.
Neutralization Test for Immunity to Poliomyelitis.
Children, Ages 5-15.

	Total	Neutralized		Failed to Neutralize	
		No.	%	No.	%
Normal children previously tested	31	13	41.9	18	58.1
Burlington	29	13	44.8	16	55.2
Bedford—No illness	28	13	46.4	15	53.6
Bedford—Suspected abortive	20	8	40.0	12	60.0

months after the occurrence of illness any immunity which might have developed as a result of such an infection should have been detected at the time of testing. From these tests it therefore appears that the widespread immunization of a population does not take place entirely at the time of an outbreak but rather in a more or less uniform manner throughout the year, or in inter-epidemic periods. This rate may, of course, vary with variations in the presence of the virus in a community.

5746

Chemical Nature of the Cyst Wall in Human Intestinal Protozoa.

CHARLES A. KOFOID, ETHEL MC NEIL AND M. J. KOPAC.

From the Department of Zoology, University of California, Berkeley.

The chemical nature of the cyst wall of human intestinal protozoa was studied on fresh material in human stools of cysts of *Endamoeba histolytica, Endolimax nana, Councilmania lafleuri,* and *C. dissimilis,* and *Giardia lamblia.*

Notably in *Councilmania lafleuri* with reagents of high osmotic pressure such as 50% H_2SO_4 and concentrated lactic acid there is at first a caving in at the pore. Later there is a return to normal contour. In lactic acid the pore is thereafter evident. In 50% HNO_3, 50% acetic acid, and 50% H_2SO_4, there is an early extension of the plug of the pore in the amoeba and in *Giardia* at the smaller end.

Various authors have referred to the cyst wall as being probably chitin, pseudo-chitin(?), or cellulose. The following tests made by us for chitin were negative: Zander's[1] iodine-zinc chloride test, Kuhnelt's[2] sulfuric acid-iodine test, and the picro-nigrosine stain. The amyloid test for cellulose was also negative.

Untreated cyst walls give a violet color reaction with Mayer's muchaematin stain. Cysts treated with dilute basic solutions to remove extraneous mucus no longer gave the mucin reaction. If the cyst wall is covered with a thin layer of mucin (secreted by the protozoan itself), the methods used to remove extraneous mucus will also remove the mucin. There is no possibility of extending this part of the investigation, since it is impossible to obtain mucus-free cysts. When the extraneous mucus and possibly the mucin is removed (use of basic solutions) there still remains the cyst wall apparently unaltered.

Ujihara[3] states that the cyst wall is possibly lipoidal in nature. The following lipoid solvents were used with negative results: ether, chloroform, alcohol, and xylol. The cysts were also unaffected by heating for 2 hours at a temperature of 140° C in 2% KCN (Topley and Wilson[4]).

---

[1] Zander, E., *Arch. f. Physiol. von Pflüger,* 1897, **66**, 545.

[2] Kuhnelt, W., *Biol. Zentralbl.,* 1928, **48**, 374.

[3] Ujihara, K., *Zeitschr. f. Hyg.,* 1914, **77**, 229.

[4] Topley, W. W. C., and Wilson, G. C., "The principles of bacteriology and immunity," 1929, **2**, 837.

The protein nature of the cyst wall was demonstrated by the positive xanthoproteic reaction; Millon's reagent also gave a faint positive reaction. We found the cyst wall to be insoluble in the following dilute acids: acetic, lactic, hydrochloric, and sulfuric. It was only soluble after boiling or a prolonged treatment in the following strong acids: hydrochloric, nitric, and sulfuric. In the strong acids a slight swelling was noticeable previous to hydrolysis. Insolubility of the cyst wall was demonstrated in the following bases: KOH, NaOH, and NH_4OH. A slight swelling was also noticeable in these reagents.

The following enzymes were used: Trypsin (Pfanstiehl), Bactotrypsin (Digestive Ferments Co.), Pepsin (Merck) (acidulated with HCl), Pancreatin (Eli Lilly & Co.), and Steapsin (Eimer & Amend). With the exception of pepsin no visible changes were evident; however in pepsin a pore was made distinctly visible.

There is no evidence to show that the cyst wall is of a carbohydrate, lipoidal, or chitinous nature. Due to the behavior of the cyst wall in the various reagents it seems only possible that the cyst wall belongs to the albuminoid or scleroprotein group. The albuminoid or scleroprotein group contains the following proteins: the keratins, fibroin, elastin, spongin, collagen, and sericin.

The following of the scleroprotein group are extremely insoluble: keratin (scales, feathers, hair, wool, horns, hoofs, nails, and silk), fibroin (substances covering the core of silk fibers), elastin, spongin (skeleton or ordinary bath sponges), and sericin (silk gelatin, forms the outer coating of the silk fiber). Since the cyst wall is extracellular, and primarily supporting or protective in function, it seems appropriate to place the cyst wall either in the group of connective tissue proteins, namely elastin, or in the group of epidermal proteins, namely, the keratins. Boiling in water and tests with acetic acid eliminate the possibility of collagen.

According to Lloyd[5] elastin does not swell in acid or basic solutions, and is rapidly digested by trypsin, while the keratins are not attacked by pepsin, trypsin, or bacterial tryptases. Keratins swell in the presence of strong basic solutions, while elastins do not exhibit this phenomenon.

According to Robertson's criteria[6] for the identification of proteins, the behavior of the cyst wall in various reagents shows evidences of great insolubility. The slight swelling of the cyst wall in basic solutions and its resistance to trypsin tends to differentiate

[5] Lloyd, D. J., "Chemistry of proteins," 1926, pp. 51-54.

[6] Robertson, T. B., "Principles of biochemistry," 1924, p. 134.

elastin from the keratins. As to the physical properties shown by micromanipulative studies the walls show flexibility as well as resilience or toughness.

Our studies of both the chemical and the physical properties of the cyst wall show that this structure has the properties of the group of keratins more nearly than those of any other group of scleroproteins. A positive test for unoxidized sulfur or for a high cystine content would convincingly demonstrate that the cyst wall is keratin; however, due to the small quantity of material present in the cyst wall, it is at present impossible to apply such tests for cystine.

5747

Digestible Constituents in Tubercle Bacillus.

WILLIAM N. BERG.

From the Berg Biological Laboratory, New York City.

In 3 investigations, Loeffler,[1] Robinovitsch,[2] and Day and Gibbs[3] found that living tubercle bacilli were killed in about 48 hours when mixed with pancreatic enzymes. According to Loeffler, the live bacilli were digested, according to Day and Gibbs they were not. None of the foregoing papers records the use of quantitative biochemical methods for detecting tubercle bacillus digestion. Nor was consideration given to the possibility that this might take place without complete dissolution of the bacterial cell.

One of the objects of the present work was to determine by quantitative methods, the extent to which tubercle bacilli undergo digestion by pancreatic enzymes acting together. In all the experiments, human and bovine strains grown on Long's synthetic agar medium were used. The nearly dry masses of bacilli were easily lifted off the agar, weighed and mixed at once with commercial pancreatic enzyme preparations.

Digestible Carbohydrate. Series of June, 1928-May, 1929. Suspensions of tubercle bacilli in chloroform Ringer solution were incubated with and without the addition of pancreatic enzyme powder. In most of the experiments sufficient chloroform and toluol was added to promptly kill the bacilli. At convenient intervals of a few

[1] Loeffler, F., *Deutsche Med. Wochenschr.*, 1913, **39**, 1025.

[2] Robinovitsch, L. G., *Endocrinology*, 1926, **10**, 602.

[3] Day, A. A., and Gibbs, W. M., *J. Infect. Dis.*, 1930, **46**, 26.

days, reducing sugar was determined in the filtrates by adaptations of Folin's[4] methods. In 30 experiments the added enzyme preparations caused an increase in reducing sugars followed by their gradual disappearance. An occasional failure to digest occurred. Calculated as dextrose, the digestible carbohydrate in 1 gm. tubercle bacilli varied up to 3 mg., depending upon the digestion period and other factors.

Digestible Fat. Series of May, 1930-June, 1931. Acid liberated from 1 gm. portions of tubercle bacilli by autolytic and by pancreatic enzymes was titrated with N/20 sodium hydroxide, with phenol red indicator. The rapid acid liberation during the first week slowed down in a month, almost to a standstill. A gram of tubercle bacilli undergoing autolysis for one month in the presence of excess of chloroform and toluol, liberated, on the average, 3.8 cc. N/20 acid. The corresponding figure is 6.5 cc., when 50 mg. of pancreatic enzyme had been added. Part of the acid liberated probably was due to protein digestion.[5] To calculate the weight of fat digested is difficult in the absence of figures for molecular weights of true glycerides in tubercle bacillus. If the above figure, 6.5 cc. N/20 acid is calculated as oleic acid, which involves many assumptions, the result indicates that all or nearly all of the fat was digested.

Physical Changes. In tubes containing the added pancreatic enzymes the bacilli seemed to swell to 2 or sometimes 3 times their original volume. When hand centrifuged for 5 minutes the flocculent bacillary masses were sedimented to their original volume. The bacilli became buttery, and sampling the mixture was impossible. In control tubes the bacilli remained the same in gross appearance although autolysis was going on. Such mixtures could be sampled.

5748

Aciduric Organisms in Dental Caries.*

RICHARD THOMPSON. (Introduced by F. P. Gay.)

From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

With the recent interest in dietary factors in dental decay the rôle

[4] Folin, O., *J. Biol. Chem.*, 1926, **70**, 410.

[5] Sherman, H. C., and Neun, D. E., *J. Am. Chem. Soc.*, 1916, **38**, 2210.

* Work supported by the Commonwealth Fund Grant for the study of the cause of Dental Caries.

of the aciduric bacteria has taken a secondary place. Attention to the possible significance of the acid-resisting, non-sporing, gram-positive bacilli was first drawn by Kligler[1] who found them constantly present on carious teeth, but irregularly on normal teeth. Since then a number of workers, Howe and Hatch,[2] McIntosh and Lazarus-Barlow,[3, 4] and Rodriguez,[5] have isolated these organisms from various depths of carious cavities and have ascribed to them major importance in the production of caries. Bunting and Palmerlee,[6] Bunting, Nickerson and Hard,[7] and Jay and Vorhees,[8] have compared the incidence of the aciduric bacilli in carious mouths and in the mouths of individuals "immune" to caries. They found the presence of these organisms almost perfectly correlated with the occurrence of caries and their absence with non-susceptibility to caries.

In the experiments reported here we have repeated and confirmed the work of the last named authors. Three groups of cases† were studied: (1) Individuals showing active and extensive caries, (2) Individuals with a history of freedom from caries and in whom careful examination revealed no trace of decay. In most cases the examination was confirmed by complete X-ray. (3) A third small group of individuals who had previously had caries but in whom no active caries had been present for some years previous to the experiment. The age distributions in the 3 groups were approximately the same: young adults being in slight preponderance in the immune group and children of school age in the caries group. Cultures obtained by swabbing the entire exposed surfaces of all the teeth were made in pH 5 chopped meat, 1% glucose infusion broth without any air seal. After 48 hours' incubation the broth was inoculated onto the surfaces of pH 7.4 1% glucose infusion

[1] Kligler, I. J., *J. Allied Dent. Soc.*, 1915, **10**, 141.

[2] Howe, Percy R., and Hatch, R. E., *Dental Cosmos*, 1917, **59**, 961.

[3] McIntosh, J., James, W. W., and Lazarus-Barlow, P., *Brit. J. Exp. Path.*, 1922, **3**, 139.

[4] McIntosh, J., James, W. W., and Lazarus-Barlow, P., *Brit. J. Exp. Path.*, 1924, **5**, 175.

[5] Rodriguez, F. E., *Milit. Dent. J.*, 1922, **5**, 199.

[6] Bunting, R. W., and Palmerlee, Faith, *J. Am. Dent. Asso.*, 1925, **12**, 381.

[7] Bunting, R. W., Nickerson, Gail, and Hard, D. G., *Dental Cosmos*, 1926, **68**, 931.

[8] Jay, P., and Vorhees, R. S., *J. Am. Dent. Assn.*, 1929, **16**, 2054.

† I am indebted to the Staff of the Columbia University School of Dentistry, especially to Drs. E. C. McBeath and L. R. Stowe, for the examination and classification of the cases.

agar plates and incubated aerobically for 48 hours. The colonies were studied with the naked eye and with the low power microscope and gram stains of the organisms were examined. Pure cultures picked from the plates were grown in pH 7.4 chopped meat, 1% glucose infusion broth.

Except for occasional yeasts and staphylococci the only organisms surviving the acid broth and developing on the plates were gram-positive, non-sporing, pleomorphic rods.

Pleomorphic gram-positive, non-sporing bacilli were obtained in great numbers from 21 of the 24 caries cases and from all 7 of those classified as arrested cases. The organisms grew profusely in the broth cultures and gave rise to numerous typical pin-point colonies on the agar plates. They were obtained from only 6 of the 19 cases classified as "immune" and in 4 of these grew only sparsely in the acid broth, producing only an occasional isolated colony when transferred to the plates. In further transfer to pH 7.4 glucose infusion broth these organisms grew profusely, indicating that their acid-resisting properties were less marked than those of the other organisms. All the colony types and morphological varieties described by Hadley, Bunting and Delves[9] were isolated; usually 1 or 2 types being obtained from any one case. There was no indication that any type or combination of types was characteristic of immune mouths or of carious mouths.

These results, although not based on a very extensive series of experiments, are very definite and completely confirm the reports of previous workers. It is difficult, however, to assay the possible significance of this degree of correlation between caries and the presence of the aciduric bacilli. The profuse growth from the 2 immune and from the 7 arrested cases indicates that the presence of the organisms is not the only factor in the production of caries. It is hardly likely that all 7 arrested cases, after years of immunity, were about to become active at the time of the cultures. It is equally difficult to believe that the degree of correlation between positive cultures and the presence of caries noted here, and by previous workers, is pure coincidence. Unless it can be shown that cavity formation offers a milieu without which aciduric bacilli of this type cannot survive (that is, in which they occupy the rôle of secondary invaders) the aciduric organisms must be included in any consideration of the etiology of dental caries.

Summary. Aciduric, gram-positive, non-sporing, pleomorphic

[9] Hadley, Faith, Bunting, R. W., and Delves, Edna A., *J. Am. Dent. Assn.*, 1930, 17, 2041.

bacilli were isolated from the mouths of 21 of 24 individuals with extensive active caries, and from 7 patients in whom caries was arrested. Of 19 individuals who had never had any dental decay, the organisms were isolated from only 6; from 4 of these, organisms were obtained which had less acid-resistance than those from the carious mouths.

5749

Blood Groups and Susceptibility to Dental Caries.*

RICHARD THOMPSON. (Introduced by F. P. Gay.)

From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

Since it is generally known that in many cases immunity to dental caries is apparently inherited, it was considered possible that this immunity or, conversely, great susceptibility might be linked with the blood group of the individual. To test this possibility bloods from a number of individuals with a history of freedom from decay and shown by dental examination to have absolutely no caries were typed. Another group of individuals in whom caries was very active and extensive was studied in the same manner.

The grouping was done in the usual way by testing the cells of the blood in question with known O, A and B sera, and the serum against known A and B cells. The tests were carried out macroscopically on a glass plate.

The distribution of the blood types in each group of cases is shown in the table.

TABLE I.

	Blood Groups				Total
	0	A	B	AB	
Immune group	11	9	3	1	24
%	45.8	37.5	12.5	4.1	
Caries group	18	12	3	3	36
%	50	33.3	8.3	8.3	

It is obvious that there is no significant difference between the distribution of blood types in the 2 groups of individuals.

* Work supported by the Commonwealth Fund Grant for the study of the cause of Dental Caries.

5750

An Attempt to Produce Mutations in Mucoraceae by Means of Ultraviolet Rays.*

BASILE J. LUYET. (Introduced by Ross G. Harrison.)

From the Osborn Zoological Laboratory, Yale University.

The possibility of producing mutations by ultraviolet rays is still under discussion. Since the indecisive results of Guyénot[1] on *Drosophila ampelophila,* experiments have been undertaken, amongst others, by Altenburg[2] on *Drosophila melanogaster,* by MacDougall[3] on *Chilodon uncinatus,* and by Stubbe[4] on *Antirrhinum majus.* While the attempts of Altenburg gave completely negative results, MacDougall described several new hereditary forms of *Chilodon* produced by ultraviolet radiation and Stubbe found a percentage of mutants almost as high with ultraviolet as obtained with X-rays, 85.71 and 87.012 respectively.

Since the spores and mycelia of the *Mucoraceae* are sensitive to a small quantity of ultraviolet radiation and present a relatively simple unicellular structure they seem particularly promising for the study of the present problem.

Ten generations of *Rhizopus nigricans* were irradiated for periods of time a little less than sufficient to kill them.

The fungus was cultivated on agarized Coon's medium in Petri dishes uncovered during exposures. The spores collected from the culture of one generation by means of a soft wet brush were spread on the surface of the medium used for the following generation. The latter was irradiated either immediately after the absorption of the water brought in by the brush or after 15 hours, the mycelia having developed. (22°C.)

I used the total radiations of an arc mercury lamp, working with 4 amperes and 60 volts on direct current. The distance between the lamp and the culture was 30 cm. The time of exposure for the spores was 35 seconds and 20 for the mycelia. (Lethal doses were 25 and 15 seconds, respectively.)[5]

* This work was done under a Seessel fellowship grant. The ultraviolet lamp was provided by Hanovia Company, through the Committee of the National Research Council on the Effects of Radiation on Living Organisms.

1 Guyénot, E., *Bull. Sc. France et Belg.,* 1914, **48**, 160.

2 Altenburg, E., *Am. Nat.,* 1928, **62**, 540.

3 MacDougall, M. S., *J. Exp. Zool.,* 1929, **54**, 95; 1931, **58**, 229.

4 Stubbe, H., *Z. Ind. Abstm. Vererb.,* 1930, **56**, 1.

5 Luyet, B., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 668.

A careful examination of the morphological and physiological behavior of the 10 irradiated generations did not show the least difference between them and the control cultures. Attention has been paid especially, besides the morphological characters described by systematists, to the germination power, the rate of growth, and the sexual behavior.

In order to have my conclusion controlled by a specialist, I sent a complete set of cultures to Miss Satina of the Carnegie Institution, who was kind enough to examine the irradiated cultures. Her conclusions were in exact keeping with mine.

In connection with these negative results I should like to emphasize a fact which, although well known, is not given sufficient attention by biologists; that is, the distance between the wave lengths of ultraviolet and X-rays is proportionately greater than that separating ultraviolet and short electric waves, and one does not usually expect the same results on living organisms from electric waves and ultraviolet radiations.

5751

Spirocheta Hispanicum (Variety Marocanum). Application in Fever Therapy and Diseases of Central Nervous System.*

FREDERICK EBERSON AND WILLIAM G. MOSSMAN.

From the Clinical and Research Laboratories, Mount Zion Hospital, San Francisco, California.

The objectionable features of malaria therapy in general paresis and neurosyphilis have suggested the use of an organism that will produce a self-limited infection for which no specific therapy is needed to cure the disease induced for therapeutic purposes. In other affections of the central nervous system, such as multiple sclerosis, dementia precox, and the like, inoculations with malaria-infected blood from another syphilitic patient are not desirable.

In the treatment of the diseases mentioned, Remlinger and Bailly,[1] Nicolle and Anderson,[2] and others have used the *Spirocheta hispani-*

* Read before Section N, Medical Sciences, June 16, 1931, at the 88th meeting of the American Association for the Advancement of Science, June 15-20, 1931, Pasadena, California.

[1] Remlinger, P., and Bailly, J., *Paris Médical*, 1930, **1**, 447.

[2] Nicolle, C., and Anderson, C., *Arch. Inst. Pasteur de Tunis*, 1930, **19**, 27.

cum (variety marocanum) with success in the production of a form of recurrent fever. The tick, *Ornithodorus marocanus,* carries a type of spirochete that is pathogenic for guinea pigs. These pigs can be readily infected by means of blood obtained from the tick, and a few drops of blood from the guinea pig in turn used to inoculate the patient by instillation into the conjunctiva or the nasal fossae.

An obvious practical advantage in artificial pyrotherapy would be gained by the use of a pure culture of a suitable infecting agent such as the *Spirocheta hispanicum.* To this end we have attempted to cultivate the organism in artificial media.

A number of ticks *(Ornithodorus marocanus)* were obtained through the courtesy of Doctors Bailly and Remlinger of the Pasteur Institute of Tunis. Various kinds of culture media were inoculated with blood from guinea pigs that had been infected with material obtained from crushed ticks and showed abundant spirochetes in blood smears stained by the Wright, Giemsa, and Romanowsky methods. The best growth was obtained in hormone broth containing brain mash prepared as follows: 1,000 gm. of fresh beef brain (sheep or calf brain may be substituted), from which blood and membranes had been removed previously, were boiled with 1,000 cc. of veal infusion, pH 8.0. The material was passed through a fine meat grinder, 0.2% glucose added, tubed and autoclaved at 15 pounds pressure for 15 minutes or sterilized in the Arnold steam sterilizer.

After 7 to 10 days' incubation at 38°C, the *Spirocheta hispanicum* was found in great numbers. Transplants to the same media resulted uniformly in excellent growth after an average incubation period of 7 days. The addition of normal human, rabbit, or guinea pig blood with or without isotonic dextrose and sodium citrate (Rous and Robertson method) was found especially desirable provided brain tissue was also present. For such experiments we employed one part minced brain media, one part isotonic dextrose (5.4% dextrose in water), one part isotonic sodium citrate (3.8% sodium citrate in water), and one part of whole blood. The organisms appeared to have a special predilection for brain tissue in keeping with a marked neurotropism that was also demonstrated by the abundance of spirochetes in the brain of inoculated guinea pigs.

Experiments thus far indicate that the organisms retain their invasive property following subculture, and, the blood of guinea pigs shows abundant spirochetes one week after inoculation with material taken from a culture. Remissions and relapses occur at intervals of 7 to 14 days in the animals and stained blood smears re-

veal the spirochetes during the relapsing stage of infection. In one animal the organisms were present in the blood stream for 7 days, absent for 2 days thereafter, and again present in large numbers for 4 successive days.

To date 18 guinea pigs and one *M. rhesus* monkey have been used in the study of the behavior of *Spirocheta hispanicum*. Blood smears have been made daily and when the organism could be demonstrated, subinoculations and cultures were attempted. The results have been uniformly successful.

In artificial culture media the spirochetes were still alive after more than 2 months' incubation at body temperature and retained the capacity to infect guinea pigs. A supply of culture material for clinical use was thus made available in the laboratory. Such specimens, if desired, can be shipped in ordinary test tubes containing the special media or in the form of capillary containers with similar material or infected blood sufficient for single or multiple inoculations.

Studies are in progress in a fairly extensive series of patients with a view to evaluate the possibilities of a wider application of the method described.

The life cycle of *Spirocheta hispanicum* has been studied and observed microscopically in the hanging drop, using blood from guinea pigs inoculated with culture or with blood from previously infected animals. The red blood corpuscles have been observed to undergo peculiar granulation changes and metamorphosis prior to the appearance of spirochetes. The organisms appeared to develop from these granules as extrusions, drawing the corpuscles along and finally breaking away from them with a rapid spinning motion and rotation along the spiral axes. In the course of one hour all stages of development could be seen by careful examination of isolated corpuscles in the field. In preparations from the blood stained by the Giemsa or Wright method the earliest changes observed were characterized by polychromatophilia and a peculiar violet or purple tinting of the blood corpuscles. This was followed by granulation with an appearance of fine or coarse stippling which in later stages showed large and coarse purple granules. These rounded bodies varied in number and size and were frequently seen to be attached to the spirochetes at different points along the axis. In the fresh as well as in the stained specimens of blood these phenomena could be observed regularly.

Remlinger and Bailly[3] have commented upon the latent period

[3] Remlinger, P., and Bailly, J., *Compt. rend. Soc. de Biol.*, 1930, **105**, 433.

following inoculation of refractory species of animals with brain emulsions obtained from infected guinea pigs. Their explanation for this prolonged incubation period was a cycle of development similar to that of rabies. Studies were not made with hanging drop preparations or stained smears. Another report of interest in connection with the life cycle is that of Hatt,[4] who studied the intestinal epithelium of Ornithodorus ticks engorged with blood from infected animals. Spirochetes with granules attached to the bodies were observed and said to be the result of fragmentation of the organisms. Such granules, it was stated, were absent on the first day and were, according to the author, due to a stage of evolution resulting from unfavorable environmental factors.

Our observations have led us to believe that the *Spirocheta hispanicum* develops in accordance with a definite life cycle in the infected red blood corpuscle, passing from a granular stage to that of the matured and typical spirochete form. We have been impressed by the added observation that fresh normal blood corpuscles, when added to artificial culture media containing the spirochetes, appeared to be infected *de novo*. These corpuscles underwent the same type of changes and cycle of spirochete development that was observed in the original preparations made with infected blood.

Further studies are in progress and cinematographic records are being prepared of the entire life cycle of this organism.

---

[4] Hatt, P., *Arch. Inst. Pasteur de Tunis*, 1929, **18**, 258.